World Journal of

Hepatology

World Journal of *Hepatology*

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

Editorial Board

2014-2017

World Journal of Hepatology

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Shuang-Teng He, MD, PhD, Professor, Research Scientist, Stroke Research Program, Kansas City VA Medical Center, Kansas City, MO 64128, United States

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 PubMed Central, PubMed, Digital Object Identifier, Directory of Open Access Journals, and Scopus.

FLYLEAF

EDITORS FOR THIS ISSUE

EDITORIAL

Three decades of hepatitis B control with vaccination

Liliane C Meireles, Rui Tato Marinho, Pierre Van Damme

Liliane C Meireles, Rui Tato Marinho, Department of Gastroenterology and Hepatology, Centro Hospitalar Lisboa Norte, 1649-035 Lisbon, Portugal

Pierre Van Damme, Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, 2610 Antwerp, Belgium

Correspondence to: Liliane C Meireles, MD, Department of Gastroenterology and Hepatology, Centro Hospitalar Lisboa Norte, Avenida Egas Moniz, 1649-035 Lisbon, Portugal. lilianeenailil@gmail.com
Telephone: +351-91-6747892
Fax: +351-21-7805610

Abstract

Hepatitis B virus (HBV) continues to represent a major health problem and can lead to acute liver failure, acute hepatitis, chronic carriership, chronic hepatitis of HBV, liver cirrhosis, liver cancer, liver transplantation and death. There is a marked difference in the geographic distribution of carriers. More than 240 million people worldwide are chronic HBV carriers. Mother-to-child transmission remains the most important mechanism of infection in countries with a high prevalence of HBV. Percutaneous/parenteral transmission and unsafe sexual practices are important mode of spread transmission of HBV in other countries. Vaccination against HBV is the gold measure for primary prevention and control of the disease. Currently, 179 countries have added HBV vaccination to their routine vaccination programs with great results. Neonatal immunization with HBV vaccine has been one of the most highly effective measures in public health and the first anti-cancer program to be launched. In this paper we review the achievements for the last three decades.

Key words: Cirrhosis; Vaccination; Primary prevention; Hepatocellular carcinoma; Hepatitis B

Core tip: It is now 50 years since the discovery of the hepatitis B virus (HBV). Effective vaccines have been available since the 80s and vaccination has proved to confer lifelong protection against hepatitis B and was highly successful in reducing the disease burden. However, the occurrence of breakthrough infections, the immunological effect of natural boosting and the effectiveness of universal hepatitis B vaccination remains a challenge. The fight against HBV is not over

yet, but the broad use of vaccination is the cornerstone and the most important measure to control HBV and all its consequences.

INTRODUCTION

Hepatitis B is a major global health problem, that can cause chronic liver disease and it is associated to a high risk of death from cirrhosis and hepatocellular carcinoma (HCC)[1,2]. Hepatitis B virus (HBV) is an oncogenic virus according World Health Organization (WHO). Roughly 30% of the world's population (more than 2 billion people) show serological evidence of current or past infection and among them 240 million are chronic HBV carriers with an incidence of 500000-700000 per year[3-8]. Adults who have had a chronic HBV infection since childhood develop HCC at a rate of 5% per decade, which is 100-300 times the rate among uninfected people[3]. HBV is a worldwide infection but there is a marked difference in the geographic distribution of carriers. Southeast Asia and Sub-Saharan Africa has one of the world's highest rates of HBV carriership ranging from 10% to 20%, while it is less than 1% in Northern Europe and America[1,3,9].

In areas of high endemicity, the infection is often acquired during the preschool years. HBV is found not only in blood but also in saliva, semen and vaginal secretions, all of which are capable of transmitting the virus. The most common route of transmission is perinatal in Asiatic countries and horizontal during childhood in African countries[3]. Other routes of transmission are transfusions of infected blood products, contaminated injections, sharing of needles among injecting drug users, unsafe sexual practices and intra-familial transmission involving non-sexual interpersonal contact over a long period of time[10,11]. It is estimated that 33% of the 16 billion annual injections administered worldwide, are unsafe, leading to approximately 20 million new HBV infections each year[12,13].

Despite advances in antiviral therapy, the primary prevention by vaccination is the gold measure of public health and the most cost-effective. Over the past 30 years, there were investments in primary prevention to increase coverage of the universal vaccination programs and consequently the herd immunity[1,12]. Due to successful vaccination programs, the epidemiology of HBV disease have been changing[14]. Thus, the burden of HBV infection for health systems can potentially be controlled through global vaccination.

VACCINATION-GLOBAL PERSPECTIVE-HISTORY OF SUCCESS

It has been 50 years since the discovery of the HBV, and, despite the availability of a prophylactic vaccine for about 30 years, HBV remains a disease of significant worldwide and global health burden[15]. For the occurrence of infection with HBV, we need: an infectious source, a susceptible host, and an established route of transmission. HBV is not entirely cytopathic; both liver damage and viral control depend on the complex interplay between virus replication and host immune response.

Humans are the only significant reservoir of HBV, so a comprehensive control strategy could eventually lead to the eradication of the virus[3]. A major obstacle to the introduction of HBV vaccination has been the high cost of HBV vaccines-but this cost has decreased due to economies of scale, local production of vaccines, competition among vaccine manufacturers, involvement of donors and bulk discounts obtained by the WHO permitting many developing countries to initiate HBV vaccine programs. The price of monovalent vaccine for developing countries has decreased from United States $3.00 per dose in 1990 to United States $0.30 per dose in 2001[16].

In the eighties, the vaccine was considered for use only in high risk individuals for acquiring HBV infection. The recognition of HBV as a serious disease burden and the availability of safe and effective HBV vaccines led WHO in 1991 to set 1997 as the target for integrating the HBV vaccine into national immunization programs worldwide[17]. WHO added a disease reduction target for HBV in 1994, calling for an 80% decrease in new HBV carrier children by 2001[1]. Progressively, it has become more widely used and recommendations for HBV vaccination have been extended to all infants in an attempt to achieve protection against HBV infection. It is the so called universal vaccination[18]. In spite of these recommendations, 6 countries in Northern Europe (Denmark, Finland, Iceland, Norway, Sweden, and the United Kingdom) have yet to implement such a policy[4,19].

Currently available HBV vaccines are extremely safe and have an efficacy of > 90% and are effective against all HBV serotypes and genotypes. The vaccination coverage is measured only after the completion of the third dose of vaccine.

In Asia, for example, HBV vaccination has been recommended for all neonates in China since 1992 and the Hepatitis B Immunization Project was initiated in 2002. The immunization coverage with three doses of vaccine increased from 71% in 2002 to 93% in 2009 among infants[20]. The surface antigen (HBsAg) prevalence in the general population decreased to 7.2% in 2006. This impact was more significant in children. In addition, the administration of the HBV vaccine have

reduced the risk of HCC among adults, nevertheless up to 10% of the adult population remain chronic carriers of HBV and prevention of HCC and cirrhosis remains a challenge for China[3,20-23].

Another history of success: prior to universal vaccination, Taiwan used to be a high endemic area for HBV, around 90% of the population aged 40 years were estimated to have been infected with HBV. At that time, about 15%-20% of the adult population was estimated to be HBV carriers. Chronic HBV is responsible for about 80% of liver cirrhosis and HCC, which are among the leading causes of mortality in Taiwan[18,24]. The vaccination of newborns of carrier mothers was implemented in 1984 and extended to all neonates in 1986. At the start of the program, the HBV carrier rate among children younger than 15 years of age was 9.8%[25]. Almost 30 years after the introduction of universal vaccination, the prevalence of HBsAg has decreased to 0.9%.

Before 1984, Alaska was an area with high HBV endemicity. However, as a consequence of the introduction of universal newborn HBV vaccination in 1984, the region was re-classified after 2000 as intermediate endemic[18,26]. Alaska is a world and happy case study for HBV vaccination and a real life history of success: all the consequences of HBV has been reduced: acute hepatitis B, chronic carriers, and HCC in children under 20 years of age.

In Africa, in the 1980s, HBsAg carriage in Gambia was 10% in children and 15% in adults. The annual incidence of HCC was 23/100000 population. The HBV vaccination was introduced gradually between 1986 and 1990. Studies conducted in 2008 have shown a vaccination coverage rate of 92%. The prevalence of HBsAg has been reduced to 1%[18].

In Europe, in 1980, in Catalonia, it is estimated that 1% of the population was chronic HBsAg carriers. Vaccination of newborns of HBsAg positive mothers and other risk populations was started in 1984. In 2002, universal HBV vaccination of children was included in the program. Between 1992 and 2010, vaccine coverage has been around 80%-90%. The reported incidence of acute HBV has fallen by 61% between 1991 and 2001[18].

The success of HBV vaccination has been clearly demonstrated. The Global Alliance for Vaccines and Immunisation recognizes that the effect of HBV vaccination in reducing the incidence of liver cancer result in an impact on public health worldwide[18]. Countries that have adopted the recommendation had a marked reduction in carrier rates as well as complications from HBV including HCC[27,28]. This has been most evident in regions with a high prevalence of chronic HBV infection. At present, global HBV vaccine coverage is estimated at 75% and has reached 91% in the Western Pacific and 89% in the American, the largest decline in incidence was seen in children[14].

TYPES OF VACCINES

The first vaccines were plasma-derived, which contained purified HBsAg obtained from the plasma of people with chronic HBV infection[4]. Derivation from plasma has left some worries regarding the potential to transmit blood infections[29]. In the following years, yeast-derived recombinant HepB vaccines have been developed by cloning the HBV S gene in yeast cells[4,30].

Currently, a mammalian cell-derived recombinant vaccine was developed. We can distinguish three vaccines of this class. One of these contain, in addition to the S antigen, antigen from the pre-S2 region while the other two contain antigens from the pre-S1 and pre-S2 regions. A controlled trial shown that this class of vaccine was associated with a better immunologic response[4]. Although this advantage, vaccines with pre-S antigens are not widely available.

Currently recombinant DNA HBV vaccines are being used for universal HBV immunization programs. Bearing in mind that more than 1 billion doses of vaccine have been used since 1982, the safety record is noteworthy[12].

HBV vaccines are not only available in monovalent formulations that protect only against hepatitis B, but also in combination formulations that protect against HBV and several other disease: diphtheria, polio, tetanus, pertussis, and *Haemophilus influenzae* type B. The immunogenicity of these multivalent vaccines is similar to that of the univalent vaccines. The multivalent vaccines are commonly used in childhood immunization programs and have greatly facilitated compliance and reduced the cost[12]. But ideally, the first dose of vaccine should be given as soon as possible after birth (< 24 h) in order to avoid early intrafamilial transmission which is around 95%. When immunizing against HBV at birth, only monovalent vaccine should be used.

Currently, WHO is evaluating the possibility to use HBV vaccines "out of the cold chain" to minimize the risk of freezing in order to improve vaccination efficacy and reduce the costs[12].

EFFICACY

Impact in complications
Hepatitis B is self-limited in most adult patients with acute infection. Meanwhile 1%-2% of patients progress to fulminant hepatic failure, and < 10% progresses to chronic infection. Chronic HBV infection can lead to liver cirrhosis, hepatic decompensation (ascites, variceal bleeding, hepatic encephalopathy, and spontaneous bacterial peritonitis), HCC, and premature death. The rate of progression from acute to chronic HBV infection is reported to be 90% in newborns and 5%-10% in adults. The progression of acute hepatitis B to chronic hepatitis is greater in Western countries. The different rates of chronicity are supposed to be attributable to the different distribution of HBV genotypes[31].

As many as 25% of HBV-infected patients will

Table 1 The proved benefits of hepatitis B vaccination
Reduction of incidence/prevalence
Acute hepatitis B
Fulminant hepatic failure
Chronic carrier
Chronic hepatitis B
Liver cirrhosis
Hepatocellular carcinoma
Comorbidities (vasculitis, neuropathy, cutaneous, personal stigma, social discrimination)

develop HCC, which is the fourth most common solid tumor worldwide. Between 500000 and 700000 people die each year from chronic infection related cirrhosis, HCC or from acute hepatitis B[12]. Reduction in the morbidity and mortality of HBV related HCC can been achieved as a result of HBV vaccination, intensive screening programs, and antiviral treatment[32-34]. The benefits of vaccination are summarized in Table 1. HBV vaccination has proven to be a safe and effective way of protecting populations from developing clinical acute or chronic HBV. The universal immunization led to a huge reduction of the prevalence of HBV and the HBV-related morbidity and mortality[6,35]. In fact HBV vaccine has been the first vaccine with a triple target: to double viral and one cancer prevention, *i.e.*, HBV, hepatitis delta and hepatocellular carcinoma. HBV is an oncogenic one, according WHO.

Response to the vaccine

A positive immune response to the vaccine is defined as the development of HBV anti-HBs at a titer of > 10 mIU/mL, after a complete and adequate immunization schedule measured preferably 1 to 3 mo after the last vaccine administration[18,36].

Long-term follow-up studies of newborn vaccination showed that antibodies become negative in 15%-50% among the vaccine responders within 5 to 10 years[4]. The decline of HBV antibody titer seemed mainly to be proportional to the antibody titer initially acquired[4]. A natural booster effect with activation of memory B cells, due to environmental exposure to HBV, can contribute to persistence of anti-HBs antibodies, particularly in areas of high endemicity. The clinical significance of the disappearance of specific antibodies in immunocompetent responders to previous vaccination remains controversial[4,18]. Long-term protection is present despite a decrease in anti-HBs antibodies over time. The exact mechanism of long-term protection, however, is not yet fully understood but it is probably due to the priming of memory cells, which are capable of producing anamnestic response when challenged. This means that the immunological memory for HBsAg can outlast antibody detection[4,36].

Protection has been estimated to persist at least for 25 years after the primary vaccination schedule[24,36-39]. Currently, decisions to offer a booster dose, based on anti-HBs antibody titre < 10 mIU/mL, is controversial

and not recommended by WHO, center for disease control or prevention or viral hepatitis prevention board. When estimating how long protection is needed, it is important to consider the periods with a high risk of exposure to HBV and increased chances for chronic evolution of an acute infection. The neonatal period and childhood constitute the high risk period, because it more likely evolves towards chronicity than infections later in life. The next high risk periods of exposure are adolescence, in which the onset of sexual activity is rising the risk of transmission.

Based on the current scientific evidence, there is consensus that there is no need to administer booster doses of vaccine to ensure long-term protection in immunocompetent subjects. A booster dose can be provided to non-responders and exceptionally to some high-risk individuals (*e.g.*, healthcare workers, couples of chronic carriers). However, more longterm data regarding the actual risk of acquiring HBV infection among individuals who completed a course of vaccination are needed before recommendations on booster dose administration can be formulated.

While most recipients of three doses of currently available HBV vaccines produce a strong, protective and long-lasting anti-HBs response, 5%-10% of healthy adults do not produce protective levels of anti-HBs, and can be considered non-responders. Several factors, such as inappropriate vaccine storage conditions, administration not following the recommendations, age, body mass index, chronic alcoholism, cirrhosis or chronic renal failure, immune-suppression, organ transplant recipients, chronic hemodialysis, type I diabetes, celiac disease and smoking, drug abuse or infections at the time of vaccination, have been found to be associated with a lower rate of response. Genome-wide association study (GWAS) has been developed to systematically investigate the associations between polymorphisms and polygenic inheritance disorders. It has been demonstrated a possible genetic predisposition to vaccine non-responsiveness likely due to the presence of specific human leukocyte antigen (HLA) haplotypes and specific single nucleotide polymorphism (rs497916, rs3922, rs676925 and rs355687) in genes of cytokine/ cytokine receptors and toll like receptors. GWAS reported that genetic variants in HLA-DP, HLA-DQ, HLA-DR influence response to vaccination[40,41].

The problem of unresponsiveness could represent - depending on the size of the problem - a global health issue, because the group of non-responders could be considered as a reservoir of HBV-susceptibility[42]. Luckily, with universal programs starting at birth or infancy, the rate of non-response very low.

Persons unresponsive to a first series of three doses of vaccine are recommended to complete a second course of vaccine. Non-responders to the second course should be evaluated for underlying chronic HBV infection. Another approach, to improve the effectiveness of vaccination, it is to administer the vaccine intradermally[41].

Vaccine escape mutants are another problem that

affects the response to the vaccine. Several mutations in the S protein have been identified, and these mutations may evade neutralizing anti-HBs and infect vaccinated people. The most widely reported escape mutants was associated with a point substitution of glycine by arginine residue at position 145 (G145R). The vaccine-escape mutants are more common in countries with high rates of endemic infection. Third generation of vaccines may also be effective in preventing infection with HBV containing a S-mutation. Nonetheless, the prevalence of these mutants appears to be low and reductions in the efficacy of HBV vaccine have not been yet observed[41,43].

CONCLUSION

For three decades HBV vaccination has effectively reduced the infection and chronicity rates and related complications. Vaccination represents the cornerstone of public health measures to control or eradicate HBV, but, other public health measures, including health education and infection control measures, remain important. The elimination of HBV is technically feasible through universal vaccination. However, we must bear in mind the hundreds of millions of already chronically infected subjects, and the 5% to 10% of individuals that do not respond to currently available vaccines[12].

Many communities have lost - in the absence of disease - the awareness of its natural consequences; the misconception emerge that vaccination is no longer required. Besides introducing universal HBV immunization programs, there is fundamental to insure that existing programs are sustained[12]. There are a few European countries, even with a substantial number of immigrants from high endemic countries, who still have not approved the inclusion of the HBV vaccine into their universal immunization programs. Healthcare workers are at risk of incidents like needle stick, sharp injuries. Occupational exposures are responsible for about 40% of HBV infection in healthcare workers. The risk of acquiring HVB infection among this special population is about 10 times higher than other group. Serologic HBV testing of at-risk health care workers remains also necessary. About 13% to 60% of individuals who have received first HBV vaccine may lose immunity[44,45]. A booster HBV vaccine could be useful for those health care workers that recognized as hyporesponders, having a titer of anti-HBs less than 100 IU/mL. In addition, health education for both health care workers and patients is still needed[10].

Vaccination and treatment strategies should take into account the risk of mutant formation. Studies on viral mutants and influence of genotype and phenotype are required[18]. Otherwise the real impact if this issue of mutants in terms of health public seems to be minimal, if any.

In conclusion, the huge benefits of HBV vaccination achieved in the last three decades, clearly superposes the other aspects. Nevertheless, much work remains to achieve the goal of global eradication of HBV infection.

REFERENCES

1   **Zhang C**, Zhong Y, Guo L. Strategies to prevent hepatitis B virus infection in China: immunization, screening, and standard medical practices. *Biosci Trends* 2013; **7**: 7-12 [PMID: 23524888]

2   **Carneiro de Moura M**, Marinho R. [Natural history and clinical manifestations of chronic hepatitis B virus]. *Enferm Infecc Microbiol Clin* 2008; **26** Suppl 7: 11-18 [PMID: 19100227]

3   **Luo Z**, Li L, Ruan B. Impact of the implementation of a vaccination strategy on hepatitis B virus infections in China over a 20-year period. *Int J Infect Dis* 2012; **16**: e82-e88 [PMID: 22178658 DOI: 10.1016/j.ijid.2011.10.009]

4   **Vitaliti G**, Praticò AD, Cimino C, Di Dio G, Lionetti E, La Rosa M, Leonardi S. Hepatitis B vaccine in celiac disease: yesterday, today and tomorrow. *World J Gastroenterol* 2013; **19**: 838-845 [PMID: 23430309 DOI: 10.3748/wjg.v19.i6.838]

5   **Trépo C**, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/s0140-6736(14)60220-8]

6   **Kao JH**, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002; **2**: 395-403 [PMID: 12127351]

7   **Van Damme P**, Ward J, Shouval D, Wiersma S, Zanetti A. Hepatitis B vaccines. In: Vaccines, 6th Edition. 2013: 205-234

8   **World Health Organization**. Prevention & Control of Viral Hepatitis Infection: Framework for Global Action, 2012. [Accessed 2014 Dec 31]. Available from: URL: http://www.who.int/csr/disease/hepatitis/en/index.html

9   **Zacharias T**, Wang W, Dao D, Wojciechowski H, Lee WM, Do S, Singal AG. HBV Outreach Programs Significantly Increase Knowledge and Vaccination Rates Among Asian Pacific Islanders. *J Community Health* 2015; **40**: 619-624 [PMID: 25476035 DOI: 10.1007/s10900-014-9975-y]

10 **Stefos A**, Gatselis N, Zachou K, Rigopoulou E, Hadjichristodoulou C, Dalekos GN. Descriptive epidemiology of chronic hepatitis B by using data from a hepatitis registry in Central Greece. *Eur J Intern Med* 2009; **20**: 35-43 [PMID: 19237090 DOI: 10.1016/j.ejim.2008.04.023]

11 **Zervou EK**, Gatselis NK, Xanthi E, Ziciadis K, Georgiadou SP, Dalekos GN. Intrafamilial spread of hepatitis B virus infection in Greece. *Eur J Gastroenterol Hepatol* 2005; **17**: 911-915 [PMID: 16093867]

12 **Lavanchy D**. Viral hepatitis: global goals for vaccination. *J Clin Virol* 2012; **55**: 296-302 [PMID: 22999800 DOI: 10.1016/j.jcv.2012.08.022]

13 **Gentile I**, Borgia G. Vertical transmission of hepatitis B virus: challenges and solutions. *Int J Womens Health* 2014; **6**: 605-611 [PMID: 24966696 DOI: 10.2147/ijwh.s51138]

14 **Kane MA**. Global status of hepatitis B immunisation. *Lancet* 1996; **348**: 696 [PMID: 8806283 DOI: 10.1016/s0140-6736(05)65598-5]

15 **Burns GS**, Thompson AJ. Viral hepatitis B: clinical and epidemiological characteristics. *Cold Spring Harb Perspect Med* 2014; **4**: a024935 [PMID: 25359547 DOI: 10.1101/cshperspect.a024935]

16 **World Health Organization**. Fact sheet N°204. [Accessed 2015 Jan 2]. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/

17 **WHO Publication**. Hepatitis B vaccines: WHO position paper--recommendations. *Vaccine* 2010; **28**: 589-590 [PMID: 19896455 DOI: 10.1016/j.vaccine.2009.10.110]

18 **FitzSimons D**, Hendrickx G, Vorsters A, Van Damme P. Hepatitis B vaccination: a completed schedule enough to control HBV lifelong? Milan, Italy, 17-18 November 2011. *Vaccine* 2013; **31**: 584-590 [PMID: 23142301 DOI: 10.1016/j.vaccine.2012.10.101]

19 **Van Damme P**, Leuridan E, Hendrickx G, Vorsters A, Theeten H, Leino T, Salminen M, Kuusi M. Should Europe have a universal hepatitis B vaccination programme? *BMJ* 2013; **347**: f4057 [PMID: 23843546 DOI: 10.1136/bmj.f4057]

20 **Yang W**, Liang X, Cui F, Li L, Hadler SC, Hutin YJ, Kane M,

Wang Y. Key outcomes and addressing remaining challenges--perspectives from a final evaluation of the China GAVI project. *Vaccine* 2013; **31** Suppl 9: J73-J78 [PMID: 24331024 DOI: 10.1016/j.vaccine.2012.09.060]

21 **Wu Z**, Cui F, Chen Y, Miao N, Gong X, Luo H, Wang F, Zheng H, Kane M, Hadler SC, Hutin YJ, Liang X, Yang W. Evaluation of immunization injection safety in China, 2010: achievements, future sustainability. *Vaccine* 2013; **31** Suppl 9: J43-J48 [PMID: 24331020 DOI: 10.1016/j.vaccine.2012.11.057]

22 **Cui F**, Luo H, Wang F, Zheng H, Gong X, Chen Y, Wu Z, Miao N, Kane M, Hennessey K, Hadler SC, Hutin YJ, Liang X, Yang W. Evaluation of policies and practices to prevent mother to child transmission of hepatitis B virus in China: results from China GAVI project final evaluation. *Vaccine* 2013; **31** Suppl 9: J36-J42 [PMID: 24331019 DOI: 10.1016/j.vaccine.2012.11.061]

23 **Kane MA**, Hadler SC, Lee L, Shapiro CN, Cui F, Wang X, Kumar R. The inception, achievements, and implications of the China GAVI Alliance Project on Hepatitis B Immunization. *Vaccine* 2013; **31** Suppl 9: J15-J20 [PMID: 24331015 DOI: 10.1016/j.vaccine.2013.03.045]

24 **Lin HH**, Wang LY, Hu CT, Huang SC, Huang LC, Lin SS, Chiang YM, Liu TT, Chen CL. Decline of hepatitis B carrier rate in vaccinated and unvaccinated subjects: sixteen years after newborn vaccination program in Taiwan. *J Med Virol* 2003; **69**: 471-474 [PMID: 12601753 DOI: 10.1002/jmv.10333]

25 **Ni YH**, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, Kao JH, Lin YC, Chen HL, Hsu HY, Chen DS. Two decades of universal hepatitis B vaccination in taiwan: impact and implication for future strategies. *Gastroenterology* 2007; **132**: 1287-1293 [PMID: 17433322 DOI: 10.1053/j.gastro.2007.02.055]

26 **McMahon BJ**, Bulkow LR, Singleton RJ, Williams J, Snowball M, Homan C, Parkinson AJ. Elimination of hepatocellular carcinoma and acute hepatitis B in children 25 years after a hepatitis B newborn and catch-up immunization program. *Hepatology* 2011; **54**: 801-807 [PMID: 21618565 DOI: 10.1002/hep.24442]

27 **Poovorawan Y**, Chongsrisawat V, Theamboonlers A, Srinivasa K, Hutagalung Y, Bock HL, Hoet B. Long-term benefit of hepatitis B vaccination among children in Thailand with transient hepatitis B virus infection who were born to hepatitis B surface antigen-positive mothers. *J Infect Dis* 2009; **200**: 33-38 [PMID: 19473096 DOI: 10.1086/599331]

28 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Evaluation of the impact of hepatitis B vaccination among children born during 1992-2005 in China. *J Infect Dis* 2009; **200**: 39-47 [PMID: 19469708 DOI: 10.1086/599332]

29 **Szmuness W**, Stevens CE, Harley EJ, Zang EA, Oleszko WR, William DC, Sadovsky R, Morrison JM, Kellner A. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980; **303**: 833-841 [PMID: 6997738 DOI: 10.1056/nejm198010093031501]

30 **Liu J**, Kosinska A, Lu M, Roggendorf M. New therapeutic vaccination strategies for the treatment of chronic hepatitis B. *Virol Sin* 2014; **29**: 10-16 [PMID: 24452539 DOI: 10.1007/s12250-014-3410-5]

31 **Michitaka K**, Hiraoka A, Tokumoto Y, Ninomiya K, Ninomiya T, Horiike N, Abe M, Hiasa Y. Clinical features of adult patients with acute hepatitis B virus infection progressing to chronic infection. *Int J Hepatol* 2014; **2014**: 358206 [PMID: 25349743 DOI: 10.1155/2014/358206]

32 **Sobeslavsky O**. Strategies for prevention and control of hepatitis B and hepatocellular carcinoma. *IARC Sci Publ* 1984; (**63**): 271-278 [PMID: 6100274]

33 **Thomas MB**, Davila M, Abbruzzese JL. Stemming the tide of hepatitis B virus related hepatocellular carcinoma? *J Clin Oncol* 2008; **26**: 172-174 [PMID: 18182657 DOI: 10.1200/jco.2007.14.4337]

34 **Zanetti A**. Hepatitis B vaccination: an important method of preventing HBV-related hepatocellular carcinoma. *Ital J Gastroenterol* 1992; **24**: 100-102 [PMID: 1315589]

35 **Romanò L**, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination. *Hum Vaccin Immunother* 2015; **11**: 53-57 [PMID: 25483515 DOI: 10.4161/hv.34306]

36 **Leuridan E**, Van Damme P. Hepatitis B and the need for a booster dose. *Clin Infect Dis* 2011; **53**: 68-75 [PMID: 21653306 DOI: 10.1093/cid/cir270]

37 **Zanetti AR**, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* 2005; **366**: 1379-1384 [PMID: 16226616 DOI: 10.1016/s0140-6736(05)67568-x]

38 **Liao SS**, Li RC, Li H, Yang JY, Zeng XJ, Gong J, Wang SS, Li YP, Zhang KL. Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine* 1999; **17**: 2661-2666 [PMID: 10418916]

39 **Yuen MF**, Lim WL, Chan AO, Wong DK, Sum SS, Lai CL. 18-year follow-up study of a prospective randomized trial of hepatitis B vaccinations without booster doses in children. *Clin Gastroenterol Hepatol* 2004; **2**: 941-945 [PMID: 15476159]

40 **Tong Hv**, Bock CT, Velavan TP. Genetic insights on host and hepatitis B virus in liver diseases. *Mutat Res Rev Mutat Res* 2014; **762**: 65-75 [PMID: 25475418 DOI: 10.1016/j.mrrev.2014.06.001]

41 **Tajiri K**, Shimizu Y. Unsolved problems and future perspectives of hepatitis B virus vaccination. *World J Gastroenterol* 2015; **21**: 7074-7083 [PMID: 26109794 DOI: 10.3748/wjg.v21.i23.7074]

42 **Schönberger K**, Riedel C, Rückinger S, Mansmann U, Jilg W, Kries RV. Determinants of Long-term protection after hepatitis B vaccination in infancy: a meta-analysis. *Pediatr Infect Dis J* 2013; **32**: 307-313 [PMID: 23249904 DOI: 10.1097/INF.0b013e31827bd1b0]

43 **Lazarevic I**. Clinical implications of hepatitis B virus mutations: recent advances. *World J Gastroenterol* 2014; **20**: 7653-7664 [PMID: 24976703 DOI: 10.3748/wjg.v20.i24.7653]

44 **Morowatishaifabad MA**, Zare Sakhvidi MJ, Gholianavval M, Masoudi Boroujeni D, Alavijeh MM. Predictors of Hepatitis B Preventive Behavioral Intentions in Healthcare Workers. *Saf Health Work* 2015; **6**: 139-142 [PMID: 26106514 DOI: 10.1016/j.shaw.2014.12.001]

45 **Sarmast Shooshtari MH**, Makvandi M, Rasti M, Neisi N, Rastegarvand N, Pouremamali A, Sadeghi Haj M, Ghaedi F. Evaluation of hepatitis B surface antibody and specific gamma interferon response in health care workers after vaccination. *Jundishapur J Microbiol* 2015; **8**: e13801 [PMID: 25789124 DOI: 10.5812/jjm.13801]

EDITORIAL

Optimal surveillance program for hepatocellular carcinoma - getting ready, but not yet

Grace Lai-Hung Wong

Grace Lai-Hung Wong, Department of Medicine and Therapeutics, 9/F Prince of Wales Hospital, the Chinese University of Hong Kong, Hong Kong, China

Grace Lai-Hung Wong, Institute of Digestive Disease, the Chinese University of Hong Kong, Hong Kong, China

Grace Lai-Hung Wong, State Key Laboratory of Digestive Disease, the Chinese University of Hong Kong, Hong Kong, China

Author contributions: Wong GLH was responsible for the conception, writing, review and revision of the manuscript.

Conflict-of-interest statement: Grace Lai-Hung Wong has served as an advisory committee member for Otsuka and Gilead. She has also served as a speaker for Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead and Otsuka.

Correspondence to: Grace Lai-Hung Wong, MD, Department of Medicine and Therapeutics, 9/F Prince of Wales Hospital, the Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, Hong Kong, China. wonglaihung@cuhk.edu.hk
Telephone: +852-26323538
Fax: +852-26373852

Abstract

Hepatocellular carcinoma (HCC) secondary to chronic viral hepatitis is a major health problem in Asian-Pacific regions due to the endemics of chronic hepatitis B and C virus infection. HCC surveillance has been recommended to patients who are at risk to develop HCC. Unfortunately, a significant proportion of patients still died in long run due to tumor recurrence. The key components of an optimal surveillance program include an accurate tumor biomarker and optimal surveillance interval. Serum alpha-fetoprotein (AFP), despite of being the most widely used biomarker for HCC surveillance, it was criticized as neither sensitive nor specific. Other HCC biomarkers, including lectin-reactive AFP (AFP-L3), des-gamma carboxyprothrombin, are still under investigations. Recent study showed cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing to be accurate with both sensitivity and specificity close to 90% in detecting HCC in a case-control study. Concerning the optimal surveillance interval, we believe one size does not fit all patients. Accurate risk prediction to assist prognostication with well-validated HCC risk scores would be useful to decide the need for HCC surveillance. These key components of an optimal HCC surveillance program should be further validated at a surveillance setting.

Key words: Antiviral therapy; Biomarkers; Hepatocellular carcinoma; Hepatocellular carcinoma risk scores; Liver stiffness measure; Surveillance program

Core tip: The key components of an optimal surveillance program include an accurate tumor biomarker and optimal surveillance interval for hepatocellular carcinoma (HCC). Cancer-associated genome-wide

hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing are two promising genomic markers of HCC. Risk prediction by HCC risk scores may assist prognostication and to decide the optimal surveillance interval.

INTRODUCTION

Hepatocellular carcinoma (HCC) secondary to chronic viral hepatitis is a major health problem in Asian-Pacific regions due to the endemics of chronic hepatitis B and C virus infection[1]. Antiviral therapy reduces the risk but does not eliminate HCC[2]. Therefore cancer surveillance remains indispensable to patients who remain at high risk despite antiviral therapy, namely those with cirrhosis[3].

BENEFITS OF HCC SURVEILLANCE

It has been recommended to offer HCC surveillance to patients who are at risk to develop HCC for almost a decade[4]. The surveillance program recommended at that time was composed of the 6-mo trans-abdominal ultrasonography and serum alpha-fetoprotein (AFP) testing. HCC surveillance improves prognosis of patients by identifying tumors of smaller sizes, fewer numbers of tumors, and longer overall survival[5]. Unfortunately, nearly 40% of patients still died in 5 years even they had received regular HCC surveillance[5]. This implies the current HCC surveillance is still far from perfect.

THE PERFECT HCC BIOMARKER - DOES IT EXIST?

The key components of the perfect surveillance program include an accurate tumor biomarker and optimal surveillance interval. Serum AFP is the most extensively applied biomarker for HCC surveillance[3]. However, its low sensitivity (20% to 65%) and specificity (50% to 94%) in discovering early HCC has resulted in the latest American guidelines abandoning AFP but using ultrasonography alone as the single surveillance tool[6]. Despite it has been recently demonstrated the high specificity of AFP in patients receiving antiviral therapy[7], it is well known that this commonly adopted tumor marker remains suboptimal.

There have been a handful of HCC biomarkers, *e.g.*, lectin-reactive AFP (AFP-L3), des-gamma carboxyprothrombin (DCP), under investigations[8]. Despite some of the biomarkers appeared promising initially, subsequent studies could not always reproduce the similar results[9]. Hence the latest European guidelines still commented that accurate tumor biomarkers for early detection of HCC needed to be developed. Such biomarkers (*i.e.*, AFP, AFP-L3 and DCP) are indeed suboptimal for routine clinical practice[3].

The dysregulated signaling pathways in HCC have been under intensive study for both diagnostic and therapeutic targets[10]. Nonetheless, HCC often involves heterogeneous pathogenesis such that multiple genes are involved (Table 1). This made using a single or a few genomic markers as HCC biomarker infeasible. Recently, cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing has been found to be accurate with both sensitivity and specificity close to 90% in detecting HCC in a case-control study[11]. The remaining issue of such genomic sequencing is that it is rather costly (approximately United States $10000 per assay). Apart from further validation of these novel genomic biomarkers in a surveillance setting, further optimization of the assay to reduce the cost yet maintaining the accuracy would be essential to make it applicable and accessible to more patients.

OPTIMAL SURVEILLANCE INTERVAL - DOES ONE SIZE FITS ALL?

There would be much economic implication in many low-to-middle economic countries if all patients at risk of HCC received antiviral therapy together with HCC surveillance. Therefore an accurate risk prediction would be able to help prognostication, deciding on the need for antiviral therapy as well as HCC surveillance. Several well-established risk factors for HBV-related HCC include advanced age, male gender, high viral load, cirrhosis. These factors are the core components of three well-validated HCC risk scores: CU-HCC[12], GAG-HCC[13] and REACH-B scores[14]. These risk scores were confirmed to be accurate in forecasting HCC up to 10 years in patients with chronic hepatitis B (CHB) who were mostly treatment-naïve.

Their validity and applicability have been recently illustrated in a large cohort of patients receiving antiviral treatment entecavir[15]. A reducion in risk scores after antiviral therapy renders to a lower risk of HCC[15]. CU-HCC score was further optimized with liver stiffness measure (LSM) by transient elastography[16]. This new LSM-HCC score excludes future HCC with high negative predictive value (99.4%-100%) at 5 years[16]. All these observations support to apply these HCC risk scores in CHB patients. Levels of care and intensities of HCC surveillance accordingly to the risk profile of patients should be offered accordingly. Patients at intermediate or high risk of HCC should receive regular HCC surveillance, despite the use of antiviral treatment[5,17].

CONCLUSION

The key components of a perfect HCC surveillance

Table 1 Major cancer genes involved in the pathogenesis of hepatocellular carcinoma

Gene	Type	Pathways
MYC	Oncogene	Proliferation control
EGF	Oncogene	EGFR signaling (mitogenic signaling)
TGFA	Oncogene	EGFR signaling (mitogenic signaling)
APC	Tumor suppressor gene	Wnt-signaling
PTEN	Tumor suppressor gene	PI3K/Akt/mTOR signaling
AKT	Oncogene	PI3K/Akt/mTOR signaling
HGF	Oncogene	Growth factor
MET	Oncogene	Growth factor receptor
PDGFR	Oncogene	Growth factor receptor
RAS	Oncogene	Ras/MAPK signaling
P53	Tumor suppressor gene	Stress response, cell cycle inhibition
E2F1	Oncogene	Cell cycle
CCND1	Oncogene	Cell cycle
Telomerase	Oncogene	Cell senescence

Modified from Zender et al[10]. EGFR: Epidermal growth factor receptor; MAPK: Mitogen-activated protein kinases; PI3K/AKT/mTOR: Phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin.

program are getting ready. They should be further validated at a surveillance setting in order to understand how exactly they can benefit our patients. Data on the cost-effectiveness of such a perfect HCC surveillance program would be useful to our policy maker. The days of HCC becoming a mostly curable disease are getting closer and closer.

REFERENCES

1   **Chan HL**, Sung JJ. Hepatocellular carcinoma and hepatitis B virus. *Semin Liver Dis* 2006; **26**: 153-161 [PMID: 16673293 DOI: 10.1055/s-2006-939753]

2   **Wong GL**, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Lai JW, Lo AO, Chan HY, Wong VW. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. *Hepatology* 2013; **58**: 1537-1547 [PMID: 23389810 DOI: 10.1002/hep.26301]

3   **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

4   **Bruix J**, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]

5   **Wong GL**, Wong VW, Tan GM, Ip KI, Lai WK, Li YW, Mak MS, Lai PB, Sung JJ, Chan HL. Surveillance programme for hepatocellular carcinoma improves the survival of patients with chronic viral hepatitis. *Liver Int* 2008; **28**: 79-87 [PMID: 17900247 DOI: 10.1111/j.1478-3231.2007.01576.x]

6   **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

7   **Wong GL**, Chan HL, Tse YK, Chan HY, Tse CH, Lo AO, Wong VW. On-treatment alpha-fetoprotein is a specific tumor marker for hepatocellular carcinoma in patients with chronic hepatitis B receiving entecavir. *Hepatology* 2014; **59**: 986-995 [PMID: 24123097 DOI: 10.1002/hep.26739]

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

9   **Bertino G**, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. *Semin Oncol* 2012; **39**: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]

10 **Zender L**, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Cancer gene discovery in hepatocellular carcinoma. *J Hepatol* 2010; **52**: 921-929 [PMID: 20385424 DOI: 10.1016/j.jhep.2009.12.034]

11 **Chan KC**, Jiang P, Chan CW, Sun K, Wong J, Hui EP, Chan SL, Chan WC, Hui DS, Ng SS, Chan HL, Wong CS, Ma BB, Chan AT, Lai PB, Sun H, Chiu RW, Lo YM. Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing. *Proc Natl Acad Sci USA* 2013; **110**: 18761-18768 [PMID: 24191000 DOI: 10.1073/pnas.1313995110]

12 **Wong VW**, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, Lui YY, Chan AT, Sung JJ, Yeo W, Chan HL, Mok TS. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. *J Clin Oncol* 2010; **28**: 1660-1665 [PMID: 20194845 DOI: 10.1200/JCO.2009.26.2675]

13 **Yuen MF**, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, But DY, Chan AO, Wong BC, Mizokami M, Lai CL. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J Hepatol* 2009; **50**: 80-88 [PMID: 18977053 DOI: 10.1016/j.jhep.2008.07.023]

14 **Yang HI**, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, Ahn SH, Chen CJ, Wong VW, Seto WK; REACH-B Working Group. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. *Lancet Oncol* 2011; **12**: 568-574 [PMID: 21497551]

15 **Wong GL**, Chan HL, Chan HY, Tse PC, Tse YK, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Wong VW. Accuracy of risk scores for patients with chronic hepatitis B receiving entecavir treatment. *Gastroenterology* 2013; **144**: 933-944 [PMID: 23415803 DOI: 10.1053/j.gastro.2013.02.002]

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

17 **Wong GL**, Wong VW. Risk prediction of hepatitis B virus-related hepatocellular carcinoma in the era of antiviral therapy. *World J Gastroenterol* 2013; **19**: 6515-6522 [PMID: 24151375 DOI: 10.3748/wjg.v19.i39.6515]

REVIEW

From the stomach to other organs: *Helicobacter pylori* and the liver

Marek Waluga, Michał Kukla, Michał Żorniak, Agata Bacik, Rafał Kotulski

Marek Waluga, Michał Kukla, Michał Żorniak, Agata Bacik, School of Medicine in Katowice, Medical University of Silesia, Department of Gastroenterology and Hepatology, 40-752 Katowice, Poland

Rafał Kotulski, Municipal Hospital, 41- 200 Sosnowiec, Poland

Author contributions: Waluga M and Żorniak M wrote the paper; Kukla M, Bacik A and Kotulski R performed the collection of the references.

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

Correspondence to: Marek Waluga, MD, PhD, School of Medicine in Katowice, Medical University of Silesia, Department of Gastroenterology and Hepatology, Medyków 18, 40-752 Katowice, Poland. mwaluga@sum.edu.pl
Telephone: +48-32-7894401
Fax: +48-32-7894402

Abstract

Many recent studies have examined the importance of *Helicobacter pylori* (*H. pylori*) infection in the pathogenesis of the diseases outside the stomach and explored the significance of this bacterium in the pathogenesis of some metabolic and cardiovascular diseases. Recent studies have provided evidence that *H. pylori* is also involved in the pathogenesis of some liver diseases. Many observations have proved that *H. pylori* infection is important in the development of insulin resistance, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver fibrosis and cirrhosis. The worsening of liver inflammation of different origins also occurs during *H. pylori* infection. Some studies have indicated that *H. pylori* infection induces autoimmunological diseases in the liver and biliary tract. The potential significance of this bacterium in carcinogenesis is unclear, but it is within the scope of interest of many studies. The proposed mechanisms through which *H. pylori* impacts the development of hepatobiliary diseases are complex and ambiguous. The importance of other *Helicobacter* species in the development of hepatobiliary diseases is also considered because they could lead to the development of inflammatory, fibrotic and necrotic injuries of the liver and, consequently, to hepatocellular carcinoma. However, many contrary viewpoints indicate that some evidence is not convincing, and further studies of the subject are needed. This review presents the current knowledge about the importance of *H. pylori* in the pathogenesis of liver and in biliary diseases.

Key words: *Helicobacter pylori*; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Liver cirrhosis; Liver fibrosis; Hepatic carcinoma

Core tip: *Helicobacter pylori* (*H. pylori*) is generally regarded as the risk factor of the development of gastric diseases, including cancer. However, some authors suggest that *H. pylori* infection can cause other disorders, including liver diseases such as non-alcoholic fatty liver diseases, non-alcoholic steatohepatitis, liver

fibrosis, cirrhosis and hepatic carcinoma. The importance of other *Helicobacter* species in the development of hepatobiliary diseases is also considered. This review examines the current knowledge on the impact of *H. pylori* infection on the pathogenesis of liver and biliary diseases and considers various points of view.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that was discovered in 1983 and was reported in 1984 in the Lancet by Warren and Marshall[1], who were awarded the Noble prize in 2005. *H. pylori* infection is very common throughout the world but is particularly common in developing countries[2]. This infection is also more common among elderly persons than adolescents[3]. As indicated by the second part of its name (*pylori*), *H. pylori* colonizes the distal part of the stomach. In most cases, the infection occurs during childhood and persists throughout life. *H. pylori* infection is the cause of many diseases, such as chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. According to the Correa theory, *H. pylori* infection causes sequential phenomena, leading from chronic gastritis through jejunal metaplasia and dysplasia to gastric cancer[4]. Many pathophysiological mechanisms are involved in the phenomena leading to inflammation and carcinogenesis. The overexpression of cyclooxygenase-2 and inducible nitric oxide synthase results in the excessive generation of prostaglandin E2 (PGE2) and nitric oxide (NO). Recent data indicate that ERK activation induced by *H. pylori* infection plays very important role in up-regulation of PGE2 (prostaglandin E2) and NO generation in the gastric mucosa at the level of inhibitory κB kinase-β and cytosolic phospholipase A2 activation. A peptide hormone, ghrelin, counters the proinflammatory consequence of the lipopolysaccharide (LPS) of *H. pylori* through Src/Act-dependent S-nitro-sylation[5]. Moreover, the ability of this hormone to counter the responses of the gastric mucosa to *H. pylori* LPS relies on phosphatidylinositol 3-kinase (PI3K) activation, which depends on the phospholipase C/protein kinase C signaling pathway. PI3K activity is required for the induction of cSrc/Act activation[6]. More detailed data on these interesting phenomena have recently been published[7].

These complicated pathophysiological mechanisms occur within the gastric mucosa. However, the chronic infection elicits not only chronic inflammatory but also immune responses on the local and systemic level[8].

This review searches for a connection between *H. pylori* infection and certain liver diseases.

H. PYLORI AND NON-ALCOHOLIC FATTY LIVER DISEASE - DOES A LINK EXIST?

Many studies indicate that *H. pylori*, the risk factor for the development of gastric diseases such as cancer, is the cause of other disorders. Some authors suggest that *H. pylori* infection and chronic liver diseases are linked[9-11]. Moreover, *H. pylori*-like DNA is more commonly found in liver samples from chronic liver disease patients than from controls[4-12].

Non-alcoholic fatty liver disease (NAFLD) is a very common disease that affects 25%-30% of the population in western countries[13,14]. Non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis are the consequences of NAFLD and influence the prevalence of morbidity and mortality. Fatty liver is significantly more often diagnosed in *H. pylori*-positive patients[9]. According to another investigation, *H. pylori* infection may be one of the hits that contributes to the pathogenesis of NAFLD, and the eradication of *H. pylori* may be significant in the treatment of this disease[11]. The pathogenic mechanism of this phenomenon is unclear.

The effect of the gut microbiota, including *H. pylori,* on liver damage has not been explored sufficiently. *Helicobacter* species may cause liver injury *via* specific toxins[15]. Moreover, invasion of *Helicobacter* in the small bowel mucosa might increase gut permeability and facilitate the passage of bacterial endotoxins *via* the portal vein to the liver[16].

H. pylori infection is positively correlated with developing metabolic syndrome and inversely correlated with morbid obesity[2,17]. The rate of seropositivity is higher in patients with metabolic syndrome than in healthy subjects[17]. However, other authors have claimed that the risk of obesity is increased after eradication of *H. pylori*. The source of this phenomenon is unclear. *H. pylori* eradication could cause an increased ghrelin concentration. Thereafter, improved appetite would lead to an increase in body mass[2,18]. Jamali *et al*[19] did not find evidence that both the reduced amount of fat in the liver and the modified lipid profile are caused by eradication therapy. There are some doubtful approaches in the methodology of this study: NAFLD was diagnosed based on ultrasound methodology. Furthermore, dyspeptic patients were included, and the control group consisted of *H. pylori* (+) patients[19]. Despite some important differences in methodology, including biopsy-proven diagnosis of NASH, selection of asymptomatic patients and *H. pylori* (-) patients in the control group, Polyzos's study obtained similar results[20]. *H. pylori* infection may be the contributing factor for NAFLD to progress to NASH. Thus, *H. pylori* eradication may be important in NASH treatment[21].

Other studies have shown that *H. pylori* infection co-exists with the development of NAFLD. The gut

microbiota may regulate insulin resistance (IR)[22]; however, such an approach is controversial. IR could be one of the important pathogenic factors. *H. pylori* infection could be involved in the pathogenesis of IR. The accumulation of free fatty acids (FFAs) in the liver is caused by a decrease in their mitochondrial β-oxidation, which is one of the feature of IR[23-25]. Whether *H. pylori* is important in the development of IR is not only unclear but also controversial. The homeostatic model of assessment IR (HOMA-IR) score is the most common method for assessing insulin sensitivity. A high HOMA-IR score indicates low insulin sensitivity. One study has shown that the HOMA-IR scores were higher in an *H. pylori*-positive group than in the negative group[26]; however, other authors have a contrary opinion[27]. *H. pylori* may be pathogenic and risk factors for obesity[28] and type 2 diabetes mellitus (DM)[29], which are components of metabolic syndrome (MS). *H. pylori* infection could explain why the pathogenesis of IR is complex[21]. The trend exists toward a positive association between *H. pylori* infection and HOMA-IR[30]. The potential association between *H. pylori* infection and IR may impact our understanding of the physiopathological mechanisms of MS, type 2 DM and NAFLD[21].

However, the pathogenesis underlying the link among *H. pylori* infection, IR and MS is unclear. Many mechanisms must be considered[31-33]. These mechanisms include the effect of fetuin-A, a glycoprotein produced by the liver[34]. Fetuin A can be an anti-inflammatory factor[35]. The level of fetuin-A is lower in *H. pylori*-infected patients compared with non-infected subjects[36]. However, other findings have shown the opposite results, indicating that *H. pylori*-infected individuals have higher fetuin-A and insulin levels and HOMA-IR scores than non-infected individuals[37]. According to other studies[38], fetuin-A has proinflammatory properties, decreases glucose tolerance and inhibits insulin receptor tyrosine kinase in the liver[33].

H. pylori infection stimulates the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8[39]. TNF-α is the important pathogenic factor in the pathogenesis of IR, NAFLD and NASH. The mechanism of TNF-α activity is complicated and includes up-regulated Ser phosphorylation[40] or inhibition of the autophosphory-lation of the tyrosyl of IRS-1[41]. The downregulation of GLUT4[42] and the acceleration of lipolysis, which increases the concentration of FFAs, is also possible. Then, these reactions evoke oxidative stress[43] and lead to detrimental effects in hepatic endoplasmic reticulum[44] and the activation of nuclear factor κB (NF-κB)[45].

Adipokines are important factors that are involved in the pathogenesis of NAFLD and NASH. They can have pro- or anti-inflammatory properties. Adiponectin is one of the first discovered adipokines. This important fat-derived compound has anti-inflammatory properties and many other ones[46], including the suppression of macrophage function, antilipogenic effects[47] and the inhibition of NF-κB activation[48]. The adiponectin level is

lower in *H. pylori*-positive patients with NAFLD than in *H. pylori* negative patients[11]. Thus, *H. pylori* infection increases the risk of NAFLD development by reducing the concentration of adiponectin.

Abnormal serum lipid compositions and lipid metabolism are very common in patients with MS and NASH. However, the impact of *H. pylori* infection on lipid metabolism is controversial. Some authors have found that the serum triglyceride level is higher in *H. pylori*-positive patients but that the high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and total cholesterol levels do not differ between *H. pylori*-positive and *H. pylori*-negative patients[49]. Others have found that *H. pylori* infection is an important factor that negatively modifies serum lipids such as increasing LDL-C and decreasing HDL-C level[36,50].

It is unclear whether the influence of *H. pylori* infection on liver steatosis and NASH has a similar pathogenesis as other species that colonizes the digestive tract. Qualitative and quantitative changes in the microbial system of the small bowel impair the intestinal barrier and bacterial translocation[51]. The increased level of endotoxin-mediated cytokines observed in patients with the portal hypertension underlies the enhanced degree of inflammation and fibrosis of the liver[52]. Small intestinal bacterial overgrowth is correlated with the severity of steatosis but not with NASH[53].

As stated above, many studies suggest that *H. pylori* infection is correlated with the spectrum of fatty liver diseases. This influence on the liver is at least partially associated with metabolic disturbances. However, the systemic recruitment of the inflammatory factors that are present at the time of *H. pylori* infection[54] could be responsible for a larger spectrum of extra-gastric manifestations, including other forms of liver damage.

H. PYLORI INFECTION AND LIVER FIBROSIS

Goo *et al*[55] showed a significant increase in the fibrotic score and aminotransferase activity in a group inoculated with *H. pylori* and CCl₄ (carbon tetrachloride) compared with a CCl₄-treated group in an animal model of fibrosis. Transforming growth factor-β1 (TGF-β1) and smooth muscle actin (α-SMA) levels were also enhanced in the co-treated group[55]. TGF-β1 is a key profibrogenic cytokine and is crucial in the production of extracellular matrix by activated hepatic stellate cells (HSCs)[56]. TGF-β1 promotes HSC differentiation into myofibroblasts[56] and facilitates the formation of α-SMA positive fibers in this cell type[57].

Carbohydrate metabolism within the liver is probably disturbed in animals inoculated with *H. pylori*. The decreased amount of hepatic glycogen is very likely result of increased glucose utilization and increased energy production from glycolysis because of mito-chondrial impairment and a depletion of the hepatic ATP

stores. Endotoxins may cause hydropic degeneration in hepatocytes[55]. Hepatocellular injury increases the serum aminotransferase activity, hydroxyproline content and extent of fibrotic area[55].

The influence of *H. pylori* infection on hepatic fibrogenesis in the absence or presence of TGF-β1 was examined in an animal model[58]. *H. pylori* strongly promoted a HSC line only if TGF-β1 was added. HSCs play pivotal roles in the progression of liver fibrosis[59]. However, TGF-β1 is essential as a fibrogenic growth factor that activates HSCs through the SMAD2/3-mediated pathway[60]. Additionally, the activation of TGF-β1 correlates with injuring factors such as oxidative stress, aging and inflammation[61].

H. PYLORI, OXIDATIVE STRESS AND LIVER CELL DAMAGE

An immunohistochemical study showed the presence of *H. pylori* antigen fragments in the liver of infected animals. A histological analysis showed that the hepatic cell architecture was disrupted, which was accompanied by slight necrosis, inflammation and ballooning of liver cells. These alterations could explain the higher vulnerability of mildly degenerated, infected liver tissue to injuring factors such as toxins, alcohol or the accumulation of fat[54-62].

The increased number of binucleated hepatocytes described by Jeong et al[63] indicates that *H. pylori* infection has additional pathological effects. The authors suggested that this phenomenon could be caused by the fusion of two hepatocytes with injured cell membranes or by regenerative processes against damage of the liver[63].

Senescence marker protein-30 (SMP30) is a multi-functional protein that prevents oxidative stress and cellular apoptosis[64,65]. Lipopolysaccharide (LPS) originating from *H. pylori* cells may underlie the oxidative stress[55]. The reduction in SMP30 in CCl4-treated livers was enhanced by *H. pylori* infection in experimental study and *H. pylori* LPS is probably its cause[55]. One of the virulence factors, vacuolating cytotoxin A (Vicar), was detected in the hepatocytes of patients with mild hypertransaminasemia and *H. pylori* infection. This finding supports the hypothesis that aminotransferase activity may be slightly elevated by cytotoxic strains of *H. pylori*[66]. According to another study, *H. pylori* is an independent factor that causes liver damage[67]. Its effective eradication leads to a decrease in aminotransferase activity in dyspeptic patients with unexplained mild hypertransaminasemia and concomitant *H. pylori* infection. This finding suggests that *H. pylori* infection is important in increasing the activity of aminotransferases[68].

H. PYLORI AND AUTOIMMUNOLOGIC LIVER DISEASES

One study has indicated that *H. pylori* infection parti-cipates in inducing autoimmunological diseases[69]. The mitochondrial autoepitopic region of pyruvate dehydrogenase complex E2 (PDC-E2) is similar to urease beta of *H. pylori*[70]. This similarity suggests that *H. pylori* infection is related to the risk of primary biliary cirrhosis (PBC). *H. pylori* DNA was detected in the livers of PBC patients[71]. However, evidence of immunological cross-activity at the CD4 T-cell and β-cell level was not found, and the importance of cross-reactive antibodies against *H. pylori* VacA antigen and human PDC-E2 was not established[69]. Moreover, the prevalence of *H. pylori* infection did not differ between PBC patients and controls[69].

Both supportive and contradictory data exist concerning a possible link between *H. pylori* infection and primary sclerosing cholangitis (PSC). Some studies detected *H. pylori* DNA in the livers of patients with PSC[72,73]. Nilsson et al[72] identified *H. pylori* and other *Helicobacter* species from patients with PSC and PBC.

PSC is often accompanied by ulcerative colitis. The hypothesis that inflammation-induced alterations in the gut flora may promote *Helicobacter* translocation from the gut to the liver in ulcerative colitis patients is very interesting. This translocation of pathogens can cause liver autoimmunity[74,75]. The prevalence of antibodies against non-gastric *H. pylori* in patients with autoimmune liver diseases is increased[69]. However, the prevalence of *H. pylori* in PSC patients did not differ compared with the prevalence in controls[69].

Evidence in support of a relationship between the prevalence of autoimmune hepatitis (AIH) and *H. pylori* infection is also insufficient[69]. A clear association between *H. pylori* seroprevalence and AIH was not confirmed in a previous study[76]. Dzierzanowska-Fangrat et al[77] evaluated pediatric patients and observed no association between *Helicobacter* infection and AIH in children. Thus, relationship between *H. pylori* colonization of gastric tissue and liver disease is controversial.

H. PYLORI INFECTION AS THE CAUSE OF LIVER CIRRHOSIS?

The prevalence of ulcers of the stomach and/or duodenum caused by *H. pylori* is higher in patients suffering from hepatic cirrhosis[78,79]. A recent meta-analysis suggests that there is also a significantly high prevalence of *H. pylori* infection among patients with cirrhosis[80]. Eradication therapy may be beneficial for cirrhotic patients because it diminishes the risk of recurrent peptic ulcers and bleeding[81,82]. However, Stalke et al[83] demonstrated a positive correlation between the degree of gastric colonization by this bacterium and parenchymatous liver damage in a group of hospitalized patients without liver cirrhosis.

H. pylori infection contributes to the development of hepatic encephalopathy and hyperammonemia[84]. In a meta-analysis of six cohort studies that involved 632 *H. pylori*-positive and 396 negative cirrhotic patients, infection was associated with elevated blood ammonia

levels[85]. Whether the *H. pylori* eradication is effective in the treatment of hepatic encephalopathy has not been fully examined.

Most data suggesting a relationship between *H. pylori* and liver complications comes from studies on viral hepatitis patients. Indeed, there have been some attempts to estimate the influence of *H. pylori* infection on the progression of fibrosis in patients with HCV-related chronic hepatitis. Esmat *et al*[86] revealed that *H. pylori* polymerase chain reaction (PCR) [cytotoxic-associated gene A (CagA)] was positive in a significantly higher percentage of patients with late fibrosis (F3 + F4 based on METAVIR staging) than with early fibrosis. In another study concerning HCV(+) patients, Queiroz *et al*[87] noted that cirrhosis in this group was associated with both age and *H. pylori*-positive status, which was confirmed by ELISA and PCR tests.

HCV patients who are coinfected with *H. pylori* have more advanced fibrosis than HCV patients without infection[88]. Decreased glycogen and total proteins in hepatocytes and cirrhotic nodules are frequently observed in HCV patients who are coinfected with *H. pylori*[88]. Additionally, *H. pylori* infection facilitates the progression to cirrhosis in patients with HCV infection[9].

In contrast, Castéra *et al*[89] did not confirm that the presence of *Helicobacter* species DNA is associated with advanced liver diseases. The lack of correlation between the presence of *H. pylori*-like DNA in the liver and positive *H. pylori* serology was also proved[89].

H. PYLORI AS AN ONCOGENIC FACTOR FOR THE LIVER?

Over-expression of hepatitis B virus (HBV) antigen (HBsAg and HBcAg) is present in the gastric mucosa of patients infected with *H. pylori* who are also suffering from hepatitis B[90]. Because both pathogens confer oncologic risk [gastric MALT-lymphoma and hepatocellular carcinoma (HCC), respectively], the authors suggest that early treatment of *H. pylori* infection could be beneficial in this group of patients[90,91].

H. pylori may be important in the development of HCC. Nilsson *et al*[92] identified *H. pylori* and similar species in liver samples from patients with HCC and cholangiocarcinoma (CCA). The presence of *H. pylori* in the bile was associated with a higher risk of CCA[93]. Additionally, *H. pylori* was detected in hepatic tissue of patients who underwent resection because of primary HCC[94,95]. Some *in vitro* studies may support the hypothesis that this bacterium may promote liver cancerogenesis. CagA *H. pylori* has an *in vitro* cytotoxic effect on HepG2 hepatocarcinoma cells[96]. Moreover, Ito *et al*[97] observed a disturbed balance between hepatic cell proliferation and apoptosis and disrupted hepatocyte replication related with persistence of intracellular *H. pylori*. However, a recent study using an animal model of hepatitis C virus-induced hepatocellular cancer showed that *H. pylori* infection does not promote the development of HCC[98].

Zhang *et al*[99] indicated that *H. pylori* exerts a pathological effect on HepG2 cells by up-regulating the expression of integrin β-1, protein kinase Cα, LIM/homeobox protein Lhx1, eIF-2-beta, MAP kinase kinase 3, PINCH protein and Ras-related protein Rab-37, which are involved in transcription, signal transduction and metabolism. This study provides indirect evidence that *H. pylori* is important for carcinogenesis in hepatic cells[99].

Xuan *et al*[100] performed a systematic review of relevant studies. Only 10 of 103 clinical trials fulfilled the very strict selection criteria and were involved in the analysis[100]. Based on this meta-analysis, the authors determined that the association of *H. pylori* infection and HCC is described by an OR of 13.63. However, these results should be interpreted with caution. *Helicobacter* DNA was detected and identified in some of the included studies as *H. pylori*-like organisms.

Persistent infection with *H. pylori* can modulate hepatocyte replication and may be important for the pathogenesis of liver diseases. Increased apoptosis could result from the release of virulent factors, which are inside the hepatocytes[97]. The virulent strain can likely arrest cell proliferation. *H. pylori* might increase the risk of TGF-β1-dependent tumorigenesis by disturbing the balance between hepatocyte apoptosis and proliferation[58]. This result is probably strain- and species-dependent[97]. Infection by *H. pylori* leads to the induction of TNF-α which also can be involved in carcinogenic processes in the liver[101].

An analysis of HCC patients indicates that 60.7% of cases are infected by *Helicobacter* species (based on PCR), and this rate is much higher ($P < 0.01$) than in control group. This finding suggests that colonization of the liver tissues by *Helicobacter* spp. could be significant for the carcinogenesis that occurs in HCC patients[100]. However, a hypothesis that explains the importance of *H. pylori* in the pathogenesis of HCC is currently lacking and requires further exploration.

The importance of *H. pylori* in the development of CCA is unclear. However, Boonyanugomol *et al*[102] found that the prevalence of *H. pylori* infection is significantly higher in patients with CCA than in patients with cholelithiasis and the control group. The authors concluded that the CagA-positive strains in particular could be involved in CCA carcinogenesis[102]. The inflammatory grade at the portal zone around the bile ducts was significantly higher in patients with CCA and *H. pylori* PCR-positive liver tissue specimens than in non-infected liver samples. Moreover, the mononuclear cell infiltration in *H. pylori* PCR-positive samples was significantly higher[102]. Inflammation leads to the production of several cytokines that can induce cell proliferation and oxidative DNA damage and decrease cell survival[103]. These phenomena could underlie the development of bile duct cancer[102]. The authors concluded that *H. pylori*, especially CagA-positive *H. pylori*, can be involved in the development of CCA. Furthermore, its importance is probably greater than that of other bacteria[102]. Fukuda

et al[104] obtained the opposite results and stated that *H. pylori* was detected in a small number of hepato-biliary cancer patients in Japan. These contradictory results suggest that *H. pylori* prevalence varies across regions[105].

The possible importance of *H. pylori* in the patho-genesis of bile duct cancer is unclear. Boonyanugomol *et al*[106] showed that the factors that were involved in *H. pylori* internalization in CCA cells were encoded by cagPAI. The polymerization of actin and α5β1-integrin is also probably important. *H. pylori* attaches to β-1 integrin receptors and can promote phosphotyrosine signaling, which activates the tyrosine phosphorylation cascade and leads to internalization of the bacterium into the hepatocytes[97]. *H. pylori* infection is also associated with proliferation, apoptosis[97,100-107] and inflammation with a concomitant increase in IL-8 in the bile ducts[106]. *H. pylori* up-regulates *NOD1* gene expression in biliary cell lines in a cagPAI-dependent manner, which is similar to the mechanism in gastric cells[106]. Moreover, increased expression levels of tall like receptor 4 (*TLR4*) and *TLR5* genes were observed in biliary cells after stimulation with *H. pylori*.

OTHER *HELICOBACTER* SPECIES AS RISK FACTORS FOR LIVER DISEASES

Based on the above observations, exploring the association between *H. pylori* infection and the course of chronic liver diseases and carcinogenesis is of interest, especially in patients with a risk of severe liver disease. Nevertheless, some evidence suggests that other *Helicobacter species* are important in certain liver pathologies. *Helicobacter hepaticus* (*H. hepaticus*) is associated with the induction of hepatic inflammation and the pathogenesis of cholestatic diseases such as PBC and PSC[108,109].

H. hepaticus was discovered in 1992. This bacterium could be crucial in the development of cholelithiasis, cholecystitis, and liver and gallbladder cancer[110]. *H. hepaticus* is similar to *H. pylori*, but it lacks virulence factors VacA and CagA. The adhesion proteins SabA, AlpA, and Bab A are also absent. However, many genes are related to *H. pylori*, including urease structural subunits, 16S rRNA, and 18 kDa immunogenic protein[110].

Kawaguchi *et al*[111] detected a microorganism that was similar to *H. pylori* in the resected gallbladder mucosa of a patient with gallstones in 1996. The possible routes of bile infection are ascending through the papillary sphincter and descending through the portal system. Thus, other bile-resistant *Helicobacter* species are found in bile juice and the gallbladder mucosa of patients with chronic cholecystitis. This phenomenon suggests that these bacterial agents could be important elements in some diseases of the biliary tract, including gallbladder cancer[111]. Currently, several dozen *Helicobacter* species have been identified, and many of them are considered as the causative agents of

various diseases of the liver and biliary tract. However, *H. pylori* can also be considered as one of the precipitating factors in the formation of the gallstones[112,113]. 16S rRNA amplification and DNA sequencing have been used to prove the presence of bacteria in gallstones. In another study, though, *Helicobacter* species were not found in bile juice. Consequently, the authors suggest that racial and demographic differences could explain these opposing results[114].

H. hepaticus infection leads to oxidative stress in the liver by increasing the level of nitrogen and oxygen active substances. This phenomenon causes carcinogenesis in the liver[115]. Particularly in patients with chronic hepatitis C virus infection, an additional infection by *H. hepaticus* may be a risk factor for the progression to liver cirrhosis or HCC[94]. Krüttgen *et al*[116] did not identify *H. hepaticus* in HCC patients with concomitant chronic hepatitis B or hepatitis C. Moreover, they also did not disprove the importance of *H. hepaticus* in HCC cases caused by other carcinogens[116].

Some experimental evidence indicates that *H. hepaticus* causes chronic liver diseases and HCC in mice. The long-term population of the liver by *H. hepaticus* leads to the development of inflammatory, fibrotic and necrotic injuries of the liver and, consequently, to HCC[117]. Proliferation and apoptosis are increased in the hepatocytes of infected mice[118].

H. hepaticus and other *Helicobacter* species, such as *H. pullorum*, *H. bilis*, may induce hepatocyte and biliary epithelia cell autoimmunity. These species can survive in low concentrations of human bile[119]. Cytolethal-distending toxin (CDT) of *H. hepaticus* plays an important role in promoting the progression of hepatitis to premalignant or dysplastic lesions in the liver and biliary tract. CDT is a multimeric cytotoxin with nuclease activity. It exposes the immune system to endogenous antigens. The activation of proinflammatory NF-κB and the increased proliferation of hepatocytes are crucial in promoting carcinogenesis[120,121]. Patients with liver diseases had increased concentrations of anti-*H. hepaticus* antibodies compared with HBV- and/or HCV-infected patients[122]. Based on a meta-analysis of 10 case-control studies in which 56% of subjects were positive for *Helicobacter spp*. infection compared with 20% in the control group, sufficient evidence supports the importance of *Helicobacter* spp. in hepatobiliary tract cancer development[123,124]. The variability of the regional prevalence of the hepatobiliary tumors indicates that the prevalence of the risk factors for this disease (*e.g.*, geographical, environmental, and genetic factors and endemic infections) is meaningful for its pathogenesis[125]. The infection rate of *Helicobacter* spp. (predominantly *H. pylori* and *H. bilis*) was significantly higher in the group with cancer of the biliary tract and in the benign biliary disease group than in the group without these diseases[125]. The risk of carcinoma development is more probable in patients with hepatitis and steatosis who are also infected with *H. hepaticus*[126].

CONCLUSION

In conclusion, *H. pylori*, which is a very important pathogenic factor in the stomach, is probably involved in the development of many other diseases. Recent studies describing its significance in cardiovascular, endocrine and metabolic diseases are complemented by investigations on its potential importance in benign or malignant diseases of other organs. Many studies indicate that *H. pylori* infection contributes to the pathogenesis of fatty liver, NAFLD and NASH. The importance of *H. pylori* in the exacerbation of inflammatory processes of different origins should also be considered. Finally, the potential importance of *H. pylori* in carcinogenic processes of the liver and biliary ducts was considered in this review. Furthermore, the significance of other *Helicobacter* species in hepatobiliary diseases was discussed. However, many opposing results indicate that some data are not convincing, and further studies are needed.

REFERENCES

1   **Fock KM**, Graham DY, Malfertheiner P. Helicobacter pylori research: historical insights and future directions. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 495-500 [PMID: 23752823 DOI: 10.1038/nrgastro.2013.96]

2   **Wu MS**, Lee WJ, Wang HH, Huang SP, Lin JT. A case-control study of association of Helicobacter pylori infection with morbid obesity in Taiwan. *Arch Intern Med* 2005; **165**: 1552-1555 [PMID: 16009873]

3   **Cizginer S**, Ordulu Z, Kadayifci A. Approach to Helicobacter pylori infection in geriatric population. *World J Gastrointest Pharmacol Ther* 2014; **5**: 139-147 [PMID: 25133042 DOI: 10.4292/wjgpt.v5.i3.139]

4   **Correa P**, Piazuelo MB. The gastric precancerous cascade. *J Dig Dis* 2012; **13**: 2-9 [PMID: 22188910 DOI: 10.1111/j.1751-2980.2011.00550.x]

5   **Slomiany BL**, Slomiany A. Induction in gastric mucosal prostaglandin and nitric oxide by Helicobacter pylori is dependent on MAPK/ERK-mediated activation of IKK-β and cPLA2: modulatory effect of ghrelin. *Inflammopharmacology* 2013; **21**: 241-251 [PMID: 23563696 DOI: 10.1007/s10787-013-0169-5]

6   **Slomiany BL**, Slomiany A. Role of ghrelin-induced phosphatidylinositol 3-kinase activation in modulation of gastric mucosal inflammatory responses to Helicobacter pylori. *Inflammopharmacology* 2014; **22**: 169-177 [PMID: 24057979 DOI: 10.1007/s10787-013-0190-8]

7   **Slomiany BL**, Slomiany A. Role of amplification in phospholipase Cγ2 activation in modulation of gastric mucosal inflammatory responses to Helicobacter pylori: effect of ghrelin. *Inflammopharmacology* 2015; **23**: 37-45 [PMID: 25362585 DOI: 10.1007/s10787-014-0220-1]

8   **Trinchieri G**. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol* 2012; **30**: 677-706 [PMID: 22224761 DOI: 10.1146/annurev-immunol-020711-075008]

9   **Doğan Z**, Filik L, Ergül B, Sarikaya M, Akbal E. Association between Helicobacter pylori and liver-to-spleen ratio: a randomized-controlled single-blind study. *Eur J Gastroenterol Hepatol* 2013; **25**: 107-110 [PMID: 23013624 DOI: 10.1097/MEG.0b013e3283590c10]

10   **Leone N**, Pellicano R, Brunello F, Cutufia MA, Berrutti M, Fagoonee S, Rizzetto M, Ponzetto A. Helicobacter pylori seroprevalence in patients with cirrhosis of the liver and hepatocellular carcinoma. *Cancer Detect Prev* 2003; **27**: 494-497 [PMID: 14642558]

11   **Polyzos SA**, Kountouras J, Papatheodorou A, Patsiaoura K, Katsiki E, Zafeiriadou E, Zavos C, Anastasiadou K, Terpos E. Helicobacter pylori infection in patients with nonalcoholic fatty liver disease. *Metabolism* 2013; **62**: 121-126 [PMID: 22841522 DOI: 10.1016/j.metabol.2012.06.007]

12   **Pirouz T**, Zounubi L, Keivani H, Rakhshani N, Hormazdi M. Detection of Helicobacter pylori in paraffin-embedded specimens from patients with chronic liver diseases, using the amplification method. *Dig Dis Sci* 2009; **54**: 1456-1459 [PMID: 18975076 DOI: 10.1007/s10620-008-0522-5]

13   **de Alwis NM**, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol* 2008; **48** Suppl 1: S104-S112 [PMID: 18304679 DOI: 10.1016/j.jhep.2008.01.009]

14   **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

15   **Taylor NS**, Fox JG, Yan L. In-vitro hepatotoxic factor in Helicobacter hepaticus, H. pylori and other Helicobacter species. *J Med Microbiol* 1995; **42**: 48-52 [PMID: 7739025]

16   **Fukuda Y**, Bamba H, Okui M, Tamura K, Tanida N, Satomi M, Shimoyama T, Nishigami T. Helicobacter pylori infection increases mucosal permeability of the stomach and intestine. *Digestion* 2001; **63 Suppl 1**: 93-96 [PMID: 11173917]

17   **Gunji T**, Matsuhashi N, Sato H, Fujibayashi K, Okumura M, Sasabe N, Urabe A. Helicobacter pylori infection is significantly associated with metabolic syndrome in the Japanese population. *Am J Gastroenterol* 2008; **103**: 3005-3010 [PMID: 19086952 DOI: 10.1111/j.1572-0241.2008.02151.x]

18   **Nwokolo CU**, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of Helicobacter pylori. *Gut* 2003; **52**: 637-640 [PMID: 12692045]

19   **Jamali R**, Mofid A, Vahedi H, Farzaneh R, Dowlatshahi S. The effect of helicobacter pylori eradication on liver fat content in subjects with non-alcoholic Fatty liver disease: a randomized open-label clinical trial. *Hepat Mon* 2013; **13**: e14679 [PMID: 24358044 DOI: 10.5812/hepatmon.14679]

20   **Polyzos SA**, Nikolopoulos P, Stogianni A, Romiopoulos I, Katsinelos P, Kountouras J. Effect of Helicobacter pylori eradication on hepatic steatosis, NAFLD fibrosis score and HSENSI in patients with nonalcoholic steatohepatitis: a MR imaging-based pilot open-label study. *Arq Gastroenterol* 2014; **51**: 261-268 [PMID: 25296089]

21   **Sumida Y**, Kanemasa K, Imai S, Mori K, Tanaka S, Shimokobe H, Kitamura Y, Fukumoto K, Kakutani A, Ohno T, Taketani H, Seko Y, Ishiba H, Hara T, Okajima A, Yamaguchi K, Moriguchi M, Mitsuyoshi H, Yasui K, Minami M, Itoh Y. Helicobacter pylori infection might have a potential role in hepatocyte ballooning in nonalcoholic fatty liver disease. *J Gastroenterol* 2015; Epub ahead of print [PMID: 25622927]

22   **Dumas ME**, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci USA* 2006; **103**: 12511-12516 [PMID: 16895997]

23   **Li M**, Shen Z, Li YM. Potential role of Helicobacter pylori infection in nonalcoholic fatty liver disease. *World J Gastroenterol* 2013; **19**: 7024-7031 [PMID: 24222944 DOI: 10.3748/wjg.v19.i41.7024]

24   **Chitturi S**, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379 [PMID: 11826411 DOI: 10.1053/jhep.2002.30692]

25   **Fong DG**, Nehra V, Lindor KD, Buchman AL. Metabolic and nutritional considerations in nonalcoholic fatty liver. *Hepatology* 2000; **32**: 3-10 [PMID: 10869282]

26   **Eshraghian A**, Hashemi SA, Hamidian Jahromi A, Eshraghian H, Masoompour SM, Davarpanah MA, Eshraghian K, Taghavi SA. Helicobacter pylori infection as a risk factor for insulin resistance.

Dig Dis Sci 2009; **54**: 1966-1970 [PMID: 19009348 DOI: 10.1007/s10620-008-0557-7]

27   **Naja F**, Nasreddine L, Hwalla N, Moghames P, Shoaib H, Fatfat M, Sibai A, Gali-Muhtasib H. Association of H. pylori infection with insulin resistance and metabolic syndrome among Lebanese adults. *Helicobacter* 2012; **17**: 444-451 [PMID: 23066847 DOI: 10.1111/j.1523-5378.2012.00970.x]

28   **Arslan E**, Atilgan H, Yavaşoğlu I. The prevalence of Helicobacter pylori in obese subjects. *Eur J Intern Med* 2009; **20**: 695-697 [PMID: 19818289 DOI: 10.1016/j.ejim.2009.07.013]

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

30   **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. The association between Helicobacter pylori infection and insulin resistance: a systematic review. *Helicobacter* 2011; **16**: 79-88 [PMID: 21435084 DOI: 10.1111/j.1523-5378.2011.00822.x]

31   **Ishibashi A**, Ikeda Y, Ohguro T, Kumon Y, Yamanaka S, Takata H, Inoue M, Suehiro T, Terada Y. Serum fetuin-A is an independent marker of insulin resistance in Japanese men. *J Atheroscler Thromb* 2010; **17**: 925-933 [PMID: 20543523]

32   **Pal D**, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med* 2012; **18**: 1279-1285 [PMID: 22842477 DOI: 10.1038/nm.2851]

33   **Srinivas PR**, Wagner AS, Reddy LV, Deutsch DD, Leon MA, Goustin AS, Grunberger G. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Mol Endocrinol* 1993; **7**: 1445-1455 [PMID: 7906861]

34   **Denecke B**, Gräber S, Schäfer C, Heiss A, Wöltje M, Jahnen-Dechent W. Tissue distribution and activity testing suggest a similar but not identical function of fetuin-B and fetuin-A. *Biochem J* 2003; **376**: 135-145 [PMID: 12943536 DOI: 10.1042/bj20030676]

35   **Wang H**, Zhang M, Bianchi M, Sherry B, Sama A, Tracey KJ. Fetuin (alpha2-HS-glycoprotein) opsonizes cationic macrophagedeactivating molecules. *Proc Natl Acad Sci USA* 1998; **95**: 14429-14434 [PMID: 9826717]

36   **Kebapcilar L**, Bilgir O, Cetinkaya E, Akyol M, Bilgir F, Bozkaya G. The effect of Helicobacter pylori eradication on macrophage migration inhibitory factor, C-reactive protein and fetuin-a levels. *Clinics* (Sao Paulo) 2010; **65**: 799-802 [PMID: 20835558]

37   **Manolakis AC**, Tiaka EK, Kapsoritakis AN, Georgoulias P, Tsiopoulos F, Valotassiou V, Potamianos SP. Increased fetuin A levels in Helicobacter pylori infection: a missing link between H. pylori and insulin resistance? *Diabetologia* 2011; **54**: 472-474 [PMID: 21116603 DOI: 10.1007/s00125-010-1995-2]

38   **Ou HY**, Yang YC, Wu HT, Wu JS, Lu FH, Chang CJ. Increased fetuin-A concentrations in impaired glucose tolerance with or without nonalcoholic fatty liver disease, but not impaired fasting glucose. *J Clin Endocrinol Metab* 2012; **97**: 4717-4723 [PMID: 23066121 DOI: 10.1210/jc.2012-2414]

39   **Basso D**, Plebani M, Kusters JG. Pathogenesis of Helicobacter pylori infection. *Helicobacter* 2010; **15** Suppl 1: 14-20 [PMID: 21054648 DOI: 10.1111/j.1523-5378.2010.00781.x]

40   **Hotamisligil GS**, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996; **271**: 665-668 [PMID: 8571133]

41   **Feinstein R**, Kanety H, Papa MZ, Lunenfeld B, Karasik A. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem* 1993; **268**: 26055-26058 [PMID: 8253716]

42   **Stephens JM**, Lee J, Pilch PF. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem* 1997; **272**: 971-976 [PMID: 8995390]

43   **Rolo AP**, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med* 2012; **52**: 59-69 [PMID: 22064361 DOI: 10.1016/j.freeradbiomed.2011.10.003]

44   **Ibrahim SH**, Kohli R, Gores GJ. Mechanisms of lipotoxicity in NAFLD and clinical implications. *J Pediatr Gastroenterol Nutr* 2011; **53**: 131-140 [PMID: 21629127 DOI: 10.1097/MPG.0b013e31822578db]

45   **Oeckinghaus A**, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat Immunol* 2011; **12**: 695-708 [PMID: 21772278]

46   **Ohashi K**, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, Pedersen AA, Kalthoff C, Tullin S, Sams A, Summer R, Walsh K. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. *J Biol Chem* 2010; **285**: 6153-6160 [PMID: 20028977 DOI: 10.1074/jbc.M109.088708]

47   **Masaki T**, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 2004; **40**: 177-184 [PMID: 15239101]

48   **Ouchi N**, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 2000; **102**: 1296-1301 [PMID: 10982546]

49   **Akbas HS**, Basyigit S, Suleymanlar I, Kemaloglu D, Koc S, Davran F, Demir I, Suleymanlar G. The assessment of carotid intima media thickness and serum paraoxonase-1 activity in Helicobacter pylori positive subjects. *Lipids Health Dis* 2010; **9**: 92 [PMID: 20804546 DOI: 10.1186/1476-511x-9-92]

50   **Satoh H**, Saijo Y, Yoshioka E, Tsutsui H. Helicobacter Pylori infection is a significant risk for modified lipid profile in Japanese male subjects. *J Atheroscler Thromb* 2010; **17**: 1041-1048 [PMID: 20610892]

51   **Korponay-Szabó IR**, Halttunen T, Szalai Z, Laurila K, Király R, Kovács JB, Fésüs L, Mäki M. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004; **53**: 641-648 [PMID: 15082580]

52   **Gäbele E**, Dostert K, Hofmann C, Wiest R, Schölmerich J, Hellerbrand C, Obermeier F. DSS induced colitis increases portal LPS levels and enhances hepatic inflammation and fibrogenesis in experimental NASH. *J Hepatol* 2011; **55**: 1391-1399 [PMID: 21703208 DOI: 10.1016/j.jhep.2011.02.035]

53   **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

54   **Buzás GM**. Metabolic consequences of Helicobacter pylori infection and eradication. *World J Gastroenterol* 2014; **20**: 5226-5234 [PMID: 24833852 DOI: 10.3748/wjg.v20.i18.5226]

55   **Goo MJ**, Ki MR, Lee HR, Yang HJ, Yuan DW, Hong IH, Park JK, Hong KS, Han JY, Hwang OK, Kim DH, Do SH, Cohn RD, Jeong KS. Helicobacter pylori promotes hepatic fibrosis in the animal model. *Lab Invest* 2009; **89**: 1291-1303 [PMID: 19736546 DOI: 10.1038/labinvest.2009.90]

56   **Tahashi Y**, Matsuzaki K, Date M, Yoshida K, Furukawa F, Sugano Y, Matsushita M, Himeno Y, Inagaki Y, Inoue K. Differential regulation of TGF-beta signal in hepatic stellate cells between acute and chronic rat liver injury. *Hepatology* 2002; **35**: 49-61 [PMID: 11786959]

57   **Dooley S**, Hamzavi J, Breitkopf K, Wiercinska E, Said HM, Lorenzen J, Ten Dijke P, Gressner AM. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rats. *Gastroenterology* 2003; **125**: 178-191 [PMID: 12851882]

58   **Ki MR**, Goo MJ, Park JK, Hong IH, Ji AR, Han SY, You SY, Lee EM, Kim AY, Park SJ, Lee HJ, Kim SY, Jeong KS. Helicobacter pylori accelerates hepatic fibrosis by sensitizing transforming growth factor-β1-induced inflammatory signaling. *Lab Invest* 2010; **90**:

1507-1516 [PMID: 20531291 DOI: 10.1038/labinvest.2010.109]

59 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090]

60 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074]

61 **Carlson ME**, Conboy MJ, Hsu M, Barchas L, Jeong J, Agrawal A, Mikels AJ, Agrawal S, Schaffer DV, Conboy IM. Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses. *Aging Cell* 2009; **8**: 676-689 [PMID: 19732043 DOI: 10.1111/j.1474-9726.2009.00517.x]

62 **Carmiel-Haggai M**, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J* 2005; **19**: 136-138 [PMID: 15522905]

63 **Jeong WI**, DO SH, Kim TH, Jeong DH, Hong IH, Ki MR, Kwak DM, Lee SS, Jee YH, Kim SB, Jeong KS. Acute effects of fast neutron irradiation on mouse liver. *J Radiat Res* 2007; **48**: 233-240 [PMID: 17536182]

64 **Lee A**, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of Helicobacter pylori infection: introducing the Sydney strain. *Gastroenterology* 1997; **112**: 1386-1397 [PMID: 9098027]

65 **Sar P**, Rath B, Subudhi U, Chainy GB, Supakar PC. Alterations in expression of senescence marker protein-30 gene by 3,3',5-triiodo-L-thyronine (T3). *Mol Cell Biochem* 2007; **303**: 239-242 [PMID: 17426926]

66 **Figura N**. Hypothesis: Helicobacter toxins and liver. *Helicobacter* 1996; **1**: 187-189 [PMID: 9398904]

67 **Tian XF**, Fan XG, Fu CY, Huang Y, Zhu C. [Experimental study on the pathological effect of Helicobacter pylori on liver tissues]. *Zhonghua Ganzangbing Zazhi* 2005; **13**: 780-783 [PMID: 16248954]

68 **Salehi H**, Minakari M, Yaghoutkar A, Tabesh E, Salehi M, Mirbagher L. The effect of Helicobacter pylori eradication on liver enzymes in patients referring with unexplained hypertransaminasemia. *Adv Biomed Res* 2014; **3**: 131 [PMID: 24949302 DOI: 10.4103/2277-9175.133256]

69 **Smyk DS**, Koutsoumpas AL, Mytilinaiou MG, Rigopoulou EI, Sakkas LI, Bogdanos DP. Helicobacter pylori and autoimmune disease: cause or bystander. *World J Gastroenterol* 2014; **20**: 613-629 [PMID: 24574735 DOI: 10.3748/wjg.v20.i3.613]

70 **Durazzo M**, Rosina F, Premoli A, Morello E, Fagoonee S, Innarella R, Solerio E, Pellicano R, Rizzetto M. Lack of association between seroprevalence of Helicobacter pylori infection and primary biliary cirrhosis. *World J Gastroenterol* 2004; **10**: 3179-3181 [PMID: 15457568]

71 **Tanaka A**, Prindiville TP, Gish R, Solnick JV, Coppel RL, Keeffe EB, Ansari A, Gershwin ME. Are infectious agents involved in primary biliary cirrhosis? A PCR approach. *J Hepatol* 1999; **31**: 664-671 [PMID: 10551390]

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

73 **Koutsoumpas A**, Mytilinaiou M, Polymeros D, Dalekos GN, Bogdanos DP. Anti-Helicobacter pylori antibody responses specific for VacA do not trigger primary biliary cirrhosis-specific antimitochondrial antibodies. *Eur J Gastroenterol Hepatol* 2009; **21**: 1220 [PMID: 19749508 DOI: 10.1097/MEG.0b013e32831a4807]

74 **Casswall TH**, Németh A, Nilsson I, Wadström T, Nilsson HO. Helicobacter species DNA in liver and gastric tissues in children and adolescents with chronic liver disease. *Scand J Gastroenterol* 2010; **45**: 160-167 [PMID: 20095882 DOI: 10.3109/00365520903426915]

75 **Eksteen B**, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hübscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004; **200**: 1511-1517 [PMID: 15557349]

76 **Durazzo M**, Pellicano R, Premoli A, Berrutti M, Leone N, Ponzetto A, Rizzetto M. Helicobacter pylori seroprevalence in patients with autoimmune hepatitis. *Dig Dis Sci* 2002; **47**: 380-383 [PMID: 11855554]

77 **Dzierzanowska-Fangrat K**, Nilsson I, Wozniak M, Jozwiak P, Rozynek E, Woynarowski M, Socha J, Ljungh A, Wadström T. Lack of an association between Helicobacter infection and autoimmune hepatitis in children. *Pol J Microbiol* 2006; **55**: 157-159 [PMID: 17419295]

78 **Ponzetto A**, Pellicano R, Leone N, Berrutti M, Turrini F, Rizzetto M. Helicobacter pylori seroprevalence in cirrhotic patients with hepatitis B virus infection. *Neth J Med* 2000; **56**: 206-210 [PMID: 10821975]

79 **Ponzetto A**, Pellicano R, Leone N, Cutufia MA, Turrini F, Grigioni WF, D'Errico A, Mortimer P, Rizzetto M, Silengo L. Helicobacter infection and cirrhosis in hepatitis C virus carriage: is it an innocent bystander or a troublemaker? *Med Hypotheses* 2000; **54**: 275-277 [PMID: 10790764]

80 **Feng H**, Zhou X, Zhang G. Association between cirrhosis and Helicobacter pylori infection: a meta-analysis. *Eur J Gastroenterol Hepatol* 2014; **26**: 1309-1319 [PMID: 25304251 DOI: 10.1097/MEG.0000000000000220]

81 **Mitrică D**, Pleşa A, Constantinescu R, Drug V, Stanciu C. Efficacy of Helicobacter pylori eradication therapy in cirrhotic patients with peptic ulcer disease. *Rev Med Chir Soc Med Nat Iasi* 2011; **115**: 367-374 [PMID: 21870725]

82 **Chang SS**, Hu HY. H. pylori eradication lower ulcers in cirrhosis. [Corrected]. *J Dig Dis* 2014; **15**: 451-458 [PMID: 24825443 DOI: 10.1111/1751-2980.12159]

83 **Stalke P**, Zółtowska A, Orłowski M, Ellert-Zygadłowska J, Witczak-Malinowska K, Michalska Z, Lakomy EA, Trocha H, Stepiński J. Correlation between liver damage and degree of gastric mucose colonization by Helicobacter pylori in subjects with parenchymatous liver damage. *Med Sci Monit* 2001; **7** Suppl 1: 271-276 [PMID: 12211735]

84 **Takuma Y**. [Helicobacter pylori infection and liver diseases]. *Gan To Kagaku Ryoho* 2011; **38**: 362-364 [PMID: 21403438]

85 **Jiang HX**, Qin SY, Min ZG, Xie MZ, Lin T, Hu BL, Guo XY. Association of Helicobacter pylori with elevated blood ammonia levels in cirrhotic patients: a meta-analysis. *Yonsei Med J* 2013; **54**: 832-838 [PMID: 23709415]

86 **Esmat G**, El-Bendary M, Zakarya S, Ela MA, Zalata K. Role of Helicobacter pylori in patients with HCV-related chronic hepatitis and cirrhosis with or without hepatocellular carcinoma: possible association with disease progression. *J Viral Hepat* 2012; **19**: 473-479 [PMID: 22676359 DOI: 10.1111/j.1365-2893.2011.01567.x]

87 **Queiroz DM**, Rocha AM, Rocha GA, Cinque SM, Oliveira AG, Godoy A, Tanno H. Association between Helicobacter pylori infection and cirrhosis in patients with chronic hepatitis C virus. *Dig Dis Sci* 2006; **51**: 370-373 [PMID: 16534683]

88 **Sakr SA**, Badrah GA, Sheir RA. Histological and histochemical alterations in liver of chronic hepatitis C patients with Helicobacter pylori infection. *Biomed Pharmacother* 2013; **67**: 367-374 [PMID: 23659901]

89 **Castéra L**, Pedeboscq A, Rocha M, Le Bail B, Asencio C, de Lédinghen V, Bernard PH, Laurent C, Lafon ME, Capdepont M, Couzigou P, Bioulac-Sage P, Balabaud C, Mégraud F, Ménard A. Relationship between the severity of hepatitis C virus-related liver disease and the presence of Helicobacter species in the liver: a prospective study. *World J Gastroenterol* 2006; **12**: 7278-7284 [PMID: 17143941]

90 **Chen NL**, Bai L, Deng T, Zhang C, Kong QY, Chen H. Expression of hepatitis B virus antigen and Helicobacter pylori infection in gastric mucosa of patients with chronic liver disease. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 223-225 [PMID: 15138114]

91 **Stolte M**, Bayerdörffer E, Morgner A, Alpen B, Wündisch T, Thiede C, Neubauer A. Helicobacter and gastric MALT lymphoma. *Gut* 2002; **50** Suppl 3: III19-III24 [PMID: 11953328]

92 **Nilsson HO**, Mulchandani R, Tranberg KG, Stenram U, Wadström T. Helicobacter species identified in liver from patients with cholangiocarcinoma and hepatocellular carcinoma. *Gastroenterology* 2001; **120**: 323-324 [PMID: 11246512]

93 **Bulajic M**, Maisonneuve P, Schneider-Brachert W, Müller P, Reischl U, Stimec B, Lehn N, Lowenfels AB, Löhr M. Helicobacter pylori and the risk of benign and malignant biliary tract disease. *Cancer* 2002; **95**: 1946-1953 [PMID: 12404289]

94 **Pellicano R**, Mazzaferro V, Grigioni WF, Cutufia MA, Fagoonee S, Silengo L, Rizzetto M, Ponzetto A. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 598-601 [PMID: 14966925]

95 **Huang Y**, Fan XG, Wang ZM, Zhou JH, Tian XF, Li N. Identification of helicobacter species in human liver samples from patients with primary hepatocellular carcinoma. *J Clin Pathol* 2004; **57**: 1273-1277 [PMID: 15563667]

96 **Chen R**, Fan XG, Huang Y, Li N, Chen CH. [In vitro cytotoxicity of Helicobacter pylori on hepatocarcinoma HepG2 cells]. *Ai Zheng* 2004; **23**: 44-49 [PMID: 14720373]

97 **Ito K**, Yamaoka Y, Yoffe B, Graham DY. Disturbance of apoptosis and DNA synthesis by Helicobacter pylori infection of hepatocytes. *Dig Dis Sci* 2008; **53**: 2532-2540 [PMID: 18253829 DOI: 10.1007/s10620-007-0163-0]

98 **García A**, Feng Y, Parry NM, McCabe A, Mobley MW, Lertpiriyapong K, Whary MT, Fox JG. Helicobacter pylori infection does not promote hepatocellular cancer in a transgenic mouse model of hepatitis C virus pathogenesis. *Gut Microbes* 2013; **4**: 577-590 [PMID: 23929035]

99 **Zhang Y**, Fan XG, Chen R, Xiao ZQ, Feng XP, Tian XF, Chen ZH. Comparative proteome analysis of untreated and Helicobacter pylori-treated HepG2. *World J Gastroenterol* 2005; **11**: 3485-3489 [PMID: 15948260]

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

101 **Rao VP**, Poutahidis T, Fox JG, Erdman SE. Breast cancer: should gastrointestinal bacteria be on our radar screen? *Cancer Res* 2007; **67**: 847-850 [PMID: 17283110]

102 **Boonyanugomol W**, Chomvarin C, Sripa B, Bhudhisawasdi V, Khuntikeo N, Hahnvajanawong C, Chamsuwan A. Helicobacter pylori in Thai patients with cholangiocarcinoma and its association with biliary inflammation and proliferation. *HPB* (Oxford) 2012; **14**: 177-184 [PMID: 22321036 DOI: 10.1111/j.1477-574.2011.00423.x]

103 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684]

104 **Fukuda K**, Kuroki T, Tajima Y, Tsuneoka N, Kitajima T, Matsuzaki S, Furui J, Kanematsu T. Comparative analysis of Helicobacter DNAs and biliary pathology in patients with and without hepatobiliary cancer. *Carcinogenesis* 2002; **23**: 1927-1931 [PMID: 12419842]

105 **Silva CP**, Pereira-Lima JC, Oliveira AG, Guerra JB, Marques DL, Sarmanho L, Cabral MM, Queiroz DM. Association of the presence of Helicobacter in gallbladder tissue with cholelithiasis and cholecystitis. *J Clin Microbiol* 2003; **41**: 5615-5618 [PMID: 14662950]

106 **Boonyanugomol W**, Chomvarin C, Hahnvajanawong C, Sripa B, Kaparakis-Liaskos M, Ferrero RL. Helicobacter pylori cag pathogenicity island (cagPAI) involved in bacterial internalization and IL-8 induced responses via NOD1- and MyD88-dependent mechanisms in human biliary epithelial cells. *PLoS One* 2013; **8**: e77358 [PMID: 24143223 DOI: 10.1371/journal.pone.0077358]

107 **Boonyanugomol W**, Chomvarin C, Song JY, Kim KM, Kim JM, Cho MJ, Lee WK, Kang HL, Rhee KH, Sripa B, Hahnvajanawong C, Baik SC. Effects of Helicobacter pylori γ-glutamyltranspeptidase on apoptosis and inflammation in human biliary cells. *Dig Dis Sci* 2012; **57**: 2615-2624 [PMID: 22581342]

108 **Kleine M**, Worbs T, Schrem H, Vondran FW, Kaltenborn A, Klempnauer J, Förster R, Josenhans C, Suerbaum S, Bektas H.

Helicobacter hepaticus induces an inflammatory response in primary human hepatocytes. *PLoS One* 2014; **9**: e99713 [PMID: 24932686 DOI: 10.1371/journal.pone.0099713]

109 **Pellicano R**, Ménard A, Rizzetto M, Mégraud F. Helicobacter species and liver diseases: association or causation? *Lancet Infect Dis* 2008; **8**: 254-260 [PMID: 18353266 DOI: 10.1016/S1473-3099(08)70066-5]

110 **Falsafi T**, Mahboubi M. Helicobacter hepaticus, a new pathogenic species of the Helicobacter genus: Similarities and differences with H. pylori. *Iran J Microbiol* 2013; **5**: 185-194 [PMID: 24475322]

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

112 **Figura N**, Cetta F, Angelico M, Montalto G, Cetta D, Pacenti L, Vindigni C, Vaira D, Festuccia F, De Santis A, Rattan G, Giannace R, Campagna S, Gennari C. Most Helicobacter pylori-infected patients have specific antibodies, and some also have H. pylori antigens and genomic material in bile: is it a risk factor for gallstone formation? *Dig Dis Sci* 1998; **43**: 854-862 [PMID: 9558044]

113 **Lee JW**, Lee DH, Lee JI, Jeong S, Kwon KS, Kim HG, Shin YW, Kim YS, Choi MS, Song SY. Identification of Helicobacter pylori in Gallstone, Bile, and Other Hepatobiliary Tissues of Patients with Cholecystitis. *Gut Liver* 2010; **4**: 60-67 [PMID: 20479914 DOI: 10.5009/gnl.2010.4.1.60]

114 **Rudi J**, Rudy A, Maiwald M, Stremmel W. Helicobacter sp. are not detectable in bile from German patients with biliary disease. *Gastroenterology* 1999; **116**: 1016-1017 [PMID: 10092334]

115 **Fox JG**, Dewhirst FE, Tully JG, Paster BJ, Yan L, Taylor NS, Collins MJ, Gorelick PL, Ward JM. Helicobacter hepaticus sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *J Clin Microbiol* 1994; **32**: 1238-1245 [PMID: 8051250]

116 **Krüttgen A**, Horz HP, Weber-Heynemann J, Vucur M, Trautwein C, Haase G, Luedde T, Roderburg C. Study on the association of Helicobacter species with viral hepatitis-induced hepatocellular carcinoma. *Gut Microbes* 2012; **3**: 228-233 [PMID: 22572832 DOI: 10.4161/gmic.19922]

117 **Rogers AB**, Fox JG. Inflammation and Cancer. I. Rodent models of infectious gastrointestinal and liver cancer. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G361-G366 [PMID: 14766534]

118 **Solnick JV**, Schauer DB. Emergence of diverse Helicobacter species in the pathogenesis of gastric and enterohepatic diseases. *Clin Microbiol Rev* 2001; **14**: 59-97 [PMID: 11148003]

119 **Lin TT**, Yeh CT, Wu CS, Liaw YF. Detection and partial sequence analysis of Helicobacter pylori DNA in the bile samples. *Dig Dis Sci* 1995; **40**: 2214-2219 [PMID: 7587792]

120 **Ge Z**, Schauer DB, Fox JG. In vivo virulence properties of bacterial cytolethal-distending toxin. *Cell Microbiol* 2008; **10**: 1599-1607 [PMID: 18489725 DOI: 10.1111/j.1462-5822.2008.01173.x]

121 **Liyanage NP**, Manthey KC, Dassanayake RP, Kuszynski CA, Oakley GG, Duhamel GE. Helicobacter hepaticus cytolethal distending toxin causes cell death in intestinal epithelial cells via mitochondrial apoptotic pathway. *Helicobacter* 2010; **15**: 98-107 [PMID: 20402812 DOI: 10.1111/j.1523-5378.210.00749.x]

122 **Fox JG**. The non-H pylori helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* 2002; **50**: 273-283 [PMID: 11788573 DOI: 10.1136/gut.50.2.273]

123 **Pandey M**, Shukla M. Helicobacter species are associated with possible increase in risk of hepatobiliary tract cancers. *Surg Oncol* 2009; **18**: 51-56 [PMID: 18715780 DOI: 10.1016/j.suronc.2008.07.002]

124 **Zhou D**, Wang JD, Weng MZ, Zhang Y, Wang XF, Gong W, Quan ZW. Infections of Helicobacter spp. in the biliary system are associated with biliary tract cancer: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 447-454 [PMID: 23470268 DOI: 10.1097/MEG.0b013e32835c0362]

125   **Segura-López FK**, Güitrón-Cantú A, Torres J. Association between Helicobacter spp. infections and hepatobiliary malignancies: a review. *World J Gastroenterol* 2015; **21**: 1414-1423 [PMID: 25663761 DOI: 10.3748/wjg.v21.i5.1414]

126   **Le Roux-Goglin E**, Dubus P, Asencio C, Jutand MA, Rosenbaum J, Mégraud F. Hepatic lesions observed in hepatitis C virus transgenic mice infected by Helicobacter hepaticus. *Helicobacter* 2013; **18**: 33-40 [PMID: 23067369 DOI: 10.1111/j.1523-5378.2012.00995.x]

REVIEW

Strategies to increase the resectability of hepatocellular carcinoma

Wong Hoi She, Kenneth SH Chok

Wong Hoi She, Kenneth SH Chok, Department of Surgery, the University of Hong Kong, Hong Kong, China

Correspondence to: Dr. Kenneth SH Chok, MBBS, Department of Surgery, the University of Hong Kong, 102 Pok Fu Lam Road, Hong Kong, China. kennethchok@gmail.com
Telephone: +852-22553025
Fax: +852-28165284

Abstract

Hepatocellular carcinoma (HCC) is best treated by liver transplantation, but the applicability of transplantation is greatly limited. Tumor resection in partial hepatectomy is hence resorted to. However, in most parts of the world, only 20%-30% of HCCs are resectable. The main reason for such a low resectability is a future liver remnant too small to be sufficient for the patient. To allow more HCC patients to undergo curative hepatectomy, a variety of ways have been developed to increase the resectability of HCC, mainly ways to increase the future liver remnants in patients through hypertrophy. They include portal vein embolization, sequential transarterial chemoembolization and portal vein embolization, staged hepatectomy, two-staged hepatectomy with portal vein ligation, and Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy. Herein we review, describe and evaluate these different ways, ways that can be life-saving.

Key words: Hepatocellular carcinoma; Hepatectomy; Portal vein ligation; Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy; Portal vein embolization

Core tip: There are different ways to increase the resectability of hepatocellular carcinoma by increasing the volume of the future liver remnant (FLR) through hypertrophy. Portal vein embolization features the the embolization of the ipsilateral side of the portal vein which supplies the liver lobe harboring the tumor, either in an open or percutaneous manner. Sequential transarterial chemoembolization and portal vein embolization is a way to augment the effect of portal vein embolization and prevent tumor progression. Staged hepatectomy is mainly for liver tumors with bilobar involvement and colorectal liver metastasis and is often aided by effective adjuvant chemotherapy. Its aim is to strike a balance between complete tumor removal and preservation of the FLR. Two-staged hepatectomy with portal vein ligation is also mainly for liver tumors with bilobar involvement and colorectal liver metastasis. In the first-stage operation, tumor in the liver portion which is designated as the FLR is cleared, and portal vein ligation is performed. The liver parenchyma is transected

only in the second-stage operation. Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy is used to speed up hypertrophy in the hope that the FLR will grow large enough for a safe hepatectomy before tumor progression occurs. It features right portal vein ligation and in-situ splitting of the intended transection surface down to the inferior vena cava. In the first-stage operation, the anterior approach is encouraged and the Pringle maneuver is discouraged, and the hilar plate is left untouched.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the most common primary liver malignancy[1,2]. Most cases of HCC in Asia are related to hepatitis B, which is prevalent in the region[3]. The best treatment for HCC is liver transplant because it removes both the tumor and the diseased liver, and a 5-year post-transplant survival rate of > 70% is expected[4-7]. Unfortunately, its applicability is limited by the shortage of liver grafts[8]. Moreover, only patients who have HCC within selection criteria (*e.g.*, the Milan criteria[9], the University of California, San Francisco criteria[10] are eligible for liver transplant. A study reported that for patients with HCC within the Milan criteria, the 5-year survival rate was 81% with living donor liver transplant and 72.8% with partial hepatectomy[11]. In the face of perpetual liver graft shortage, hepatectomy remains an important curative measure as it can achieve a satisfactory survival outcome.

Hepatectomy has been evolving and is getting more technically challenging as surgeons are pushing limits. They are trying to operate on HCCs larger and larger and with more and more nodules, but a R0 resection is always the ultimate goal. The applicability of hepatectomy is often limited by an inadequate future liver remnant (FLR) or a marginal liver, especially in patients with underlying hepatitis or cirrhosis. The success of hepatectomy depends on many factors, which include status of the tumor, the patient's clinical status and underlying liver function, and the size of the FLR of the patient[12-14]. Aggressive hepatectomy may still be beneficial for patients who have advanced HCC with large or multiple tumors or intrahepatic venous invasion if they are properly selected[15]. Curative hepatectomy is the first-line treatment for HCC at many centers. The resectability of HCC often rises with the volume of the FLR, and therefore different measures are employed for increasing such volume. Moreover, a larger FLR would probably mean better overall and disease-free survival.

SURGICAL RESECTION

In the management of HCC, liver resection for tumor clearance is the first-line curative treatment for patients with preserved liver function[11,14,16]. Major hepatectomy can be performed safely nowadays with careful patient selection[12,14], better understanding of the liver anatomy[17], improvement of surgical techniques, and advances of surgical instruments. Widely adopted techniques include the hanging maneuver[18], the anterior approach for avoidance of mobilization and rupture of large tumors[19,20], the Pringle maneuver[21], and meticulous control of central venous pressure for reduction of reduce blood loss[22]. Widely employed instruments include Cavitron Ultrasound Surgical Aspirator, hydrojet[23,24], the Harmonic scalpel, LigaSure, Harmonic Ace, and Thunderbeat[25]. Although complications and perioperative mortalities still occur, the rates are acceptable[13,14,26,27]. However, major hepatectomy may not be suitable for patients with marginal liver function or a relatively small FLR. The University of Hong Kong uses indocynaine green clearance test as an important tool to assess their patients' preoperative liver function[28]. Unfortunately, there is no perfect test for the prediction of postoperative mortality[29,30]. For risk stratification for major hepatectomy, usually a combination of assessment modalities is adopted, which usually includes measurement of the disease's Child-Pugh grading, indocynaine green clearance test, renal function test by creatinine level check, and platelet count.

Location of tumors is a decisive factor in surgical planning. The amount of liver removed in hepatectomy decides the volume of the liver remnant. Major hepatectomy can only be offered to patients with an adequate FLR and adequate post-resection liver function. To avoid massive bleeding and vascular insult to the liver, preservation or reconstruction of major hepatic veins in addition to meticulous surgical skills is needed[29]. A patient's liver volume can be measured by tracing the liver contour in the cross-sectional image on computed tomography volumetry[31], and a patient's standard liver volume can be derived from his weight and height with different formulae[32,33]. The volume of his FLR can then be calculated. Patients with cirrhosis have relatively poor liver function, and thus need a larger FLR[34-37] to lower the risk of liver failure. At The University of Hong Kong, for patients who have Child-Pugh A cirrhosis and an indocynaine green retention rate $\leqslant$ 20% at 15 min, an FLR > 30% of the estimated standard liver volume is preferred for right hepatectomy, and an FLR > 35% of the estimated standard liver volume is preferred for extended right hepatectomy or right trisectionectomy. Patients who have cirrhosis and an inadequate FLR have a high risk of post-hepatectomy liver failure[37,38].

REGENERATION OF LIVER REMNANT

Different types of injury (*e.g.*, ischemia/reperfusion, resection) will induce a hypertrophic response called

the atrophy-hypertrophy complex in a liver remnant. Hypertrophy is simultaneously caused by increased endothelial shear stress, hepatocellular swelling, and activated growth factors/cytokines due to increased portal flow[39]. The idea of portal vein embolization (PVE) is to occlude a liver segment or lobule so as to bring about its ischemia[40,41] and consequent atrophy, thereby inducing hypertrophy of the part of liver not atrophied.

PVE

PVE is indicated for patients who are considered for right or extended right hepatectomy but with a relatively small FLR. By PVE, the size of an FLR can be increased. PVE features the embolization of the ipsilateral side of the portal vein which supplies the liver lobe harboring the tumor, either in an open or percutaneous manner, thereby inducing hypertrophy of the FLR[42,43]. To date, there is still no straight value on the minimum volume of an FLR which allows major hepatectomy to be performed safely. An FLR > 35% of the estimated standard liver volume has been recommended for patients with cirrhosis, steatosis, or chronic hepatitis[28,36,37,44-48]. PVE is rarely required before extended left hepatectomy or left trisectionectomy, since the right posterior section usually constitutes about 30% of the total liver volume[49,50]. The technique for embolizing the segment-4 portal vein is crucial; if the vein is not properly blocked, suboptimal hypertrophy may results.

Liver volume assessment after PVE

The FLR volume will be reassessed 4-8 wk after PVE[51,52]. Rapid growth of the FLR is anticipated in the first 3-4 wk. Generally, an 8%-30% enlargement over 2-6 wk is expected[43,52-55]. Hypertrophy is usually slower in the presence of cirrhosis[56]. Studies comparing major hepatectomy with and without preceding PVE reported that comparable and even superior long-term outcomes were achieved with PVE[45,57-63]. With PVE, patients who would have been considered inoperable in the past because of their small FLR have the option of hepatectomy with reasonable long-term surgical outcomes.

Complications of PVE

PVE can be performed in an open or percutaneous manner. Open right portal vein ligation often renders the subsequent surgery difficult due to vascular or fibrotic adhesions around the hilar structure. Open transileocolic PVE is performed with cannulation of the ileocolic vein in addition to embolization of the right portal vein in an antegrade manner or percutaneous portal vein cannulation and retrograde embolization. Ipsilateral percutaneous PVE is generally preferred because of the low invasiveness and an easier access to segment-4 portal vein branches[64-66]. Different ways of PVE all carry a risk of complication, such as main portal vein thrombosis. Prompt surgical intervention or

anticoagulation is needed if the embolic agent crosses the contralateral side of the portal vein, which would cause liver failure in the case of bilateral PVE, resulting in death[67]. Hemorrhage or catastrophic bleeding at the puncture site may also occur, which also requires prompt surgical intervention. In addition, PVE induces inflammatory response near the hilar structure, which may increase the difficulty in dissection in the subsequent hepatectomy and raise the surgical risk.

SEQUENTIAL TRANSARTERIAL CHEMOEMBOLIZATION AND PVE

PVE can be given to HCC patients with underlying cirrhosis, but hepatic regeneration and thus hypertrophy of the FLR would be impaired in the presence of cirrhosis[68-70]. On the other hand, it is likely that the arterial flow increases compensatorily in segments with PVE, thereby stimulating tumor progression as HCC is a hypervascular tumor supplied by the hepatic artery blood flow[71-73]. To augment the effect of PVE and prevent tumor progression, the treatment sequential transarterial chemoembolization and PVE is used[57]. Studies comparing patients who received this treatment and patients who did not found that patients who did showed a higer rate of hypertrophy of FLR and a bigger increase in their FLR[57,58], and the rate of tumor progression was lower as tumor necrosis was evident[74]. This treatment is not without risk; it could cause ischemic parenchymal damage[75], but overall, it is feasible and safe, and it allows HCC patients who would otherwise be denied hepatectomy to undergo curative resection with reasonable postoperative 5-year overall and disease-free survival[57,58,76].

STAGED HEPATECTOMY

Staged hepatectomy is mainly for HCC with bilobar involvement and colorectal liver metastasis, and is often aided by effective adjuvant chemotherapy[77-79]. In staged hepatectomy, two or more planned hepatectomies are performed at different time to achieve a R0 resection. It is distinguished from unplanned repeat hepatectomies for recurrent disease[80]. Its aim is to strike a balance between complete tumor removal and preservation of the FLR. The chance of postoperative liver failure can be reduced if bilobar tumors are removed in a staged manner. The preserved portion of the liver should be relatively spared by the disease with sufficient FLR and adequate vascular inflow and outflow[81]. However, there is always the chance that the tumor tissue is cut across during the first-stage procedure, resulting in tumor spillage and peritoneal metastasis, and rendering the planned second-stage procedure unfeasible. Besides, tumors may grow despite temporary chemotherapy during the hepatic regenerative period, which may also preclude further operation. Repeat resection is technically demanding, as not only all the dissection
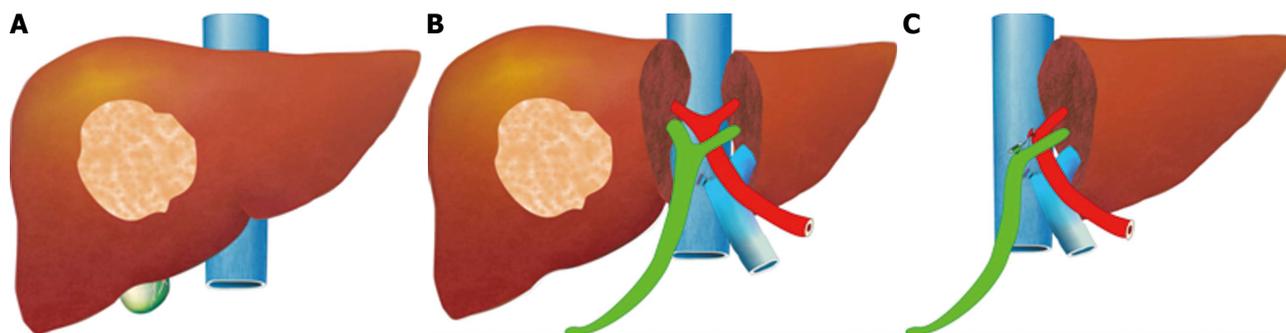
Figure 1 Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy. A: Tumor in the right liver lobe, the future liver remnant (the left liver lobe) will be small; B: In stage-1 ALPPS, the right portal vein is ligated, liver transected, inferior vena cava exposed, and gallbladder resected; C: In stage-2 ALPPS, the left liver lobe has hypertrophied, the right lobe with tumor is resected, right hepatic artery and right hepatic duct transected and ligated. Green: Bile duct; Red: Hepatic artery; Blue: Posterior inferior vena cava and anterior portal vein. ALPPS: Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy.

planes have already been disturbed, adhesiolysis can also be very difficult. Adhesiolysis near the liver hilum and the inferior vena cava is particularly challenging, as massive bleeding may occur. Staged hepatectomy for HCC was not common[79]; it was mostly for colorectal liver metastasis[78,82-85].

TWO-STAGED HEPATECTOMY WITH PORTAL VEIN LIGATION

This treatment requires two laparotomies and is also mainly for HCC with bilobar involvement and colorectal liver metastasis. In the first laparotomy, tumor in the liver portion which is designated as the FLR is cleared, and portal vein ligation is performed. Other required resection such as that of colorectal primary tumor is also done in the first laparotomy. The liver parenchyma is transected only in the second laparotomy but not in the first. The portal vein ligation is to induce hypertrophy of the FLR, allowing hepatectomy in the second-stage procedure and decreasing the risk of postoperative liver failure. Portal vein ligation has been found to be as effective as PVE[86]. However, open portal vein ligation poses the risk of adhesion formation over the hilum, which may increase the difficulty of dissection in the second-stage operation.

ASSOCIATING LIVER PARTITION AND PORTAL VEIN LIGATION IN STAGED HEPATECTOMY

For hepatectomy, one of the limiting factors is inadequate volume of the FLR. Although the aforesaid methods are effective in inducing hypertrophy of the FLR, it takes several weeks for it to reach a satisfactory volume[43]. Tumor progression may occur before the FLR is large enough for the planned hepatectomy to be conducted. If a major vessel such as the ipsilateral portal vein is invaded by tumor, the tumor will progress in terms of days and contralateral deposition and metastasis of the tumor will occur, rendering the tumor inoperable[72,73,87].

Associating Liver Partition and Portal Vein Ligation in Staged Hepatectomy (ALPPS) is one of the main surgical innovations in recent years. The procedure, which was invented by chance, was initially carried out by Dr. Hans Schlitt from Germany in an intended extended right hepatectomy for hilar cholangiocarcinoma[88]. In the surgery, palliative left hepaticojejunostomy was performed because the FLR was small, with division of the liver parenchyma along the falciform ligament and ligation of the right portal vein. On day 8 after the surgery, computed tomography was performed. To Dr. Schlitt's surprise, the left lateral section had grown enormously in size. The diseased portion of the liver was subsequently removed in another surgery. This novel technique was later termed "ALPPS"[89]. The idea of ALPPS is to speed up hypertrophy of the FLR (the left lobe or the left lateral section) by right portal vein ligation and in-situ splitting of the intended transection surface down to the inferior vena cava (Figure 1). Generally, the FLR regenerates to a volume adequate for a safe hepatectomy in days.

ALPPS was initially applied to relatively normal livers, such as in the case of colorectal liver metastasis. Later it was also applied to livers with steatosis or cirrhosis[88,90-94]. A 70% increase in FLR volume has been reported[95]. ALPPS is better than conventional PVE when the rate and the percentage of hypertrophy are concerned[96,97]. The shorter the interval between the two operations is, the less mature the adhesions would be, and hence the second operation would also be easier.

Most of the reported cases of ALPPS are on non-cirrhotic livers, and there has not been any report on the rate of hypertrophy in cirrhotic livers. However, one would anticipate that some patients would not have adequate hypertrophy of the contralateral side and hence the second stage operation is not possible. ALPPS carries certain risks. The right hepatic artery could be injured, and liver failure could occur after right portal vein ligation. The Pringle maneuver would pose a further risk of liver injury and is thus not recommended. In the first-stage operation, adoption of the anterior approach allows liver transection without mobilization of the right

lobe, thereby minimizing adhesion formation[98], and the hilar plate is left untouched so as to minimize the chance of biliary complication. Bile leakage from the transection surface can result in biloma and increases the chance of infection and thus the risk of sepsis, which may forbid the second-stage operation. ALPPS is very technically challenging and demanding, and therefore should not be carried out by inexperienced surgeons.

Indications for ALPPS

ALPPS should be carried out with a curative intent. It is indicated for patients who have a large tumor load and a marginal FLR[96], even with tumor invasion of major vessels, such as the portal vein[92]. ALPPS renders some inoperable tumors potentially operable.

Morbidity and mortality after ALPPS

Complication and mortality are inevitable with any surgery; ALPPS is no exception. Perioperative mortality rates of 12%-28% have been reported, which are overall higher than those of conventional major hepatectomy[95,96,99,100]. A complication rate high at 50% has been recorded[99,101]. Complications include ascites, bile leakage, persisting cholestasis and sepsis, wound infection, and other inflammatory and infective complications. ALPPS increases operability at the price of a heightened morbidity and mortality. Keeping morbidity and mortality at the minimum requires careful patient selection, meticulous surgical technique, and accurate decision as to proceeding to the second-stage operation or not.

The long-term outcome of ALPPS is still unknown. Long-term overall survival and disease-free survival are still pending. Further studies as well as input from different centers are required but not yet available. However, ALPPS has improved the operative rate, and it is hoped that it will improve the overall and disease-free survival of patients. Nonetheless, larger trials are needed to document its efficacy especially for those patient with HCC and background cirrhosis.

CONCLUSION

There are revolutional changes of surgical methods to increase the resectability of HCCs, and various ways to increase the volume of the FLR of patients considered for major hepatectomy have been developed. Improvement in surgical techniques also allows patients to benefit from surgical resection with safety. Treatment modalities are always evolving for the better. Hopefully, ALPPS will continue to develop and long-term results will be available in the near future.

REFERENCES

1   **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/S0140-6736(03)14964-1]
2   **Kim do Y**, Han KH. Epidemiology and surveillance of hepato-

cellular carcinoma. *Liver Cancer* 2012; **1**: 2-14 [PMID: 24159567 DOI: 10.1159/000339016]
3   **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
4   **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
5   **Lee KK**, Kim DG, Moon IS, Lee MD, Park JH. Liver transplantation versus liver resection for the treatment of hepatocellular carcinoma. *J Surg Oncol* 2010; **101**: 47-53 [PMID: 19798686 DOI: 10.1002/jso.21415]
6   **Ito T**, Takada Y, Ueda M, Haga H, Maetani Y, Oike F, Ogawa K, Sakamoto S, Ogura Y, Egawa H, Tanaka K, Uemoto S. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007; **13**: 1637-1644 [PMID: 18044766 DOI: 10.1002/lt.21281]
7   **Tamura S**, Sugawara Y, Kokudo N. Living donor liver transplantation for hepatocellular carcinoma: the Japanese experience. *Oncology* 2011; **81** Suppl 1: 111-115 [PMID: 22212944 DOI: 10.1159/000333270]
8   **Lo CM**, Fan ST, Liu CL, Chan SC, Wong J. The role and limitation of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 440-447 [PMID: 15004774 DOI: 10.1002/lt.20097]
9   **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
10 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
11 **Fan ST**, Poon RT, Yeung C, Lam CM, Lo CM, Yuen WK, Ng KK, Liu CL, Chan SC. Outcome after partial hepatectomy for hepatocellular cancer within the Milan criteria. *Br J Surg* 2011; **98**: 1292-1300 [PMID: 21656513 DOI: 10.1002/bjs.7583]
12 **Poon RT**, Fan ST. Assessment of hepatic reserve for indication of hepatic resection: how I do it. *J Hepatobiliary Pancreat Surg* 2005; **12**: 31-37 [PMID: 15754097 DOI: 10.1007/s00534-004-0945-0]
13 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified? *Ann Surg* 2002; **236**: 602-611 [PMID: 12409666 DOI: 10.1097/01.SLA.0000033038.38956.5E]
14 **Fan ST**, Mau Lo C, Poon RT, Yeung C, Leung Liu C, Yuen WK, Ming Lam C, Ng KK, Ching Chan S. Continuous improvement of survival outcomes of resection of hepatocellular carcinoma: a 20-year experience. *Ann Surg* 2011; **253**: 745-758 [PMID: 21475015 DOI: 10.1097/SLA.0b013e3182111195]
15 **Yau T**, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* 2014; **146**: 1691-700.e3 [PMID: 24583061 DOI: 10.1053/j.gastro.2014.02.032]
16 **Cauchy F**, Soubrane O, Belghiti J. Liver resection for HCC: patient's selection and controversial scenarios. *Best Pract Res Clin Gastroenterol* 2014; **28**: 881-896 [PMID: 25260315 DOI: 10.1016/j.bpg.2014.08.013]
17 **Couinaud C**. Liver anatomy: portal (and suprahepatic) or biliary segmentation. *Dig Surg* 1999; **16**: 459-467 [PMID: 10805544 DOI: 10.1159/000018770]
18 **Belghiti J**, Guevara OA, Noun R, Saldinger PF, Kianmanesh R. Liver hanging maneuver: a safe approach to right hepatectomy

without liver mobilization. *J Am Coll Surg* 2001; **193**: 109-111 [PMID: 11442247 DOI: 10.1016/S1072-7515(01)00909-7]

19   **Liu CL**, Fan ST, Lo CM, Tung-Ping Poon R, Wong J. Anterior approach for major right hepatic resection for large hepatocellular carcinoma. *Ann Surg* 2000; **232**: 25-31 [PMID: 10862191 DOI: 10.1097/00000658-200007000-00004]

20   **Fan ST**, Liu CL. Anterior approach for major right hepatic resection. *J Hepatobiliary Pancreat Surg* 2005; **12**: 356-361 [PMID: 16258802 DOI: 10.1007/s00534-005-1000-5]

21   **Man K**, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Prospective evaluation of Pringle maneuver in hepatectomy for liver tumors by a randomized study. *Ann Surg* 1997; **226**: 704-711; discussion 711-713 [PMID: 9409569 DOI: 10.1097/00000658-199712000-00007]

22   **Melendez JA**, Arslan V, Fischer ME, Wuest D, Jarnagin WR, Fong Y, Blumgart LH. Perioperative outcomes of major hepatic resections under low central venous pressure anesthesia: blood loss, blood transfusion, and the risk of postoperative renal dysfunction. *J Am Coll Surg* 1998; **187**: 620-625 [PMID: 9849736 DOI: 10.1016/S1072-7515(98)00240-3]

23   **Lesurtel M**, Selzner M, Petrowsky H, McCormack L, Clavien PA. How should transection of the liver be performed?: a prospective randomized study in 100 consecutive patients: comparing four different transection strategies. *Ann Surg* 2005; **242**: 814-22, discussion 822-3 [PMID: 16327491 DOI: 10.1097/01.sla.0000189121.35617.d7]

24   **Takayama T**, Makuuchi M, Kubota K, Harihara Y, Hui AM, Sano K, Ijichi M, Hasegawa K. Randomized comparison of ultrasonic vs clamp transection of the liver. *Arch Surg* 2001; **136**: 922-928 [PMID: 11485528 DOI: 10.1001/archsurg.136.8.922]

25   **Milsom J**, Trencheva K, Monette S, Pavoor R, Shukla P, Ma J, Sonoda T. Evaluation of the safety, efficacy, and versatility of a new surgical energy device (THUNDERBEAT) in comparison with Harmonic ACE, LigaSure V, and EnSeal devices in a porcine model. *J Laparoendosc Adv Surg Tech A* 2012; **22**: 378-386 [PMID: 22364404 DOI: 10.1089/lap.2011.0420]

26   **Fan ST**, Lai EC, Lo CM, Ng IO, Wong J. Hospital mortality of major hepatectomy for hepatocellular carcinoma associated with cirrhosis. *Arch Surg* 1995; **130**: 198-203 [PMID: 7848092 DOI: 10.1001/archsurg.1995.01430020088017]

27   **Fan ST**, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* 1999; **229**: 322-330 [PMID: 10077043 DOI: 10.1097/00000658-199903000-00004]

28   **Lau H**, Man K, Fan ST, Yu WC, Lo CM, Wong J. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg* 1997; **84**: 1255-1259 [PMID: 9313707 DOI: 10.1002/bjs.1800840917]

29   **Fan ST**. Liver functional reserve estimation: state of the art and relevance for local treatments: the Eastern perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 380-384 [PMID: 19865790 DOI: 10.1007/s00534-009-0229-9]

30   **Manizate F**, Hiotis SP, Labow D, Roayaie S, Schwartz M. Liver functional reserve estimation: state of the art and relevance for local treatments: the Western perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 385-388 [PMID: 19936599 DOI: 10.1007/s00534-009-0228-x]

31   **Heymsfield SB**, Fulenwider T, Nordlinger B, Barlow R, Sones P, Kutner M. Accurate measurement of liver, kidney, and spleen volume and mass by computerized axial tomography. *Ann Intern Med* 1979; **90**: 185-187 [PMID: 443650 DOI: 10.7326/0003-4819-90-2-185]

32   **Chan SC**, Lo CM, Chok KS, Sharr WW, Cheung TT, Tsang SH, Chan AC, Fan ST. Validation of graft and standard liver size predictions in right liver living donor liver transplantation. *Hepatol Int* 2011; **5**: 913-917 [PMID: 21484120 DOI: 10.1007/s12072-011-9264-0]

33   **Urata K**, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, Momose Y, Komiyama A, Makuuchi M. Calculation of child and adult standard liver volume for liver transplantation.

34   **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]

35   **Vauthey JN**, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C, Hicks M, Alsfasser G, Lauwers G, Hawkins IF, Caridi J. Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. *Surgery* 2000; **127**: 512-519 [PMID: 10819059 DOI: 10.1067/msy.2000.105294]

36   **Kubota K**, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 1997; **26**: 1176-1181 [PMID: 9362359 DOI: 10.1053/jhep.1997.v26.pm0009362359]

37   **Shirabe K**, Shimada M, Gion T, Hasegawa H, Takenaka K, Utsunomiya T, Sugimachi K. Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume. *J Am Coll Surg* 1999; **188**: 304-309 [PMID: 10065820 DOI: 10.1016/S1072-7515(98)00301-9]

38   **Schindl MJ**, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ. 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]

39   **Kim RD**, Kim JS, Watanabe G, Mohuczy D, Behrns KE. Liver regeneration and the atrophy-hypertrophy complex. *Semin Intervent Radiol* 2008; **25**: 92-103 [PMID: 21326550 DOI: 10.1055/s-2008-1076679]

40   **Lemasters JJ**, Ji S, Thurman RG. Centrilobular injury following hypoxia in isolated, perfused rat liver. *Science* 1981; **213**: 661-663 [PMID: 7256265 DOI: 10.1126/science.7256265]

41   **Jungermann K**, Kietzmann T. Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* 2000; **31**: 255-260 [PMID: 10655244 DOI: 10.1002/hep.510310201]

42   **Kinoshita H**, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. *World J Surg* 1986; **10**: 803-808 [PMID: 3022488 DOI: 10.1007/BF01655244]

43   **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57 [PMID: 18156923 DOI: 10.1097/SLA.0b013e31815f6e5b]

44   **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]

45   **Palavecino M**, Chun YS, Madoff DC, Zorzi D, Kishi Y, Kaseb AO, Curley SA, Abdalla EK, Vauthey JN. Major hepatic resection for hepatocellular carcinoma with or without portal vein embolization: Perioperative outcome and survival. *Surgery* 2009; **145**: 399-405 [PMID: 19303988 DOI: 10.1016/j.surg.2008.10.009]

46   **Ribero D**, Curley SA, Imamura H, Madoff DC, Nagorney DM, Ng KK, Donadon M, Vilgrain V, Torzilli G, Roh M, Vauthey JN. Selection for resection of hepatocellular carcinoma and surgical strategy: indications for resection, evaluation of liver function, portal vein embolization, and resection. *Ann Surg Oncol* 2008; **15**: 986-992 [PMID: 18236112 DOI: 10.1245/s10434-007-9731-y]

47   **Mullin EJ**, Metcalfe MS, Maddern GJ. How much liver resection is too much? *Am J Surg* 2005; **190**: 87-97 [PMID: 15972178 DOI: 10.1016/j.amjsurg.2005.01.043]

48   **Yanaga K**, Honda H, Ikeda Y, Nishizaki AT, Yamamoto K, Sugimachi K. Significance of liver size in hepatic surgery. *HPB Surg* 1997; **10**: 195-199; discussion 199-200 [PMID: 9184872 DOI: 10.1155/1997/34842]

49   **Abdalla EK**, Denys A, Chevalier P, Nemr RA, Vauthey JN. Total and segmental liver volume variations: implications for liver

Hepatology 1995; **21**: 1317-1321 [PMID: 7737637 DOI: 10.1002/hep.1840210515]

surgery. *Surgery* 2004; **135**: 404-410 [PMID: 15041964 DOI: 10.1016/j.surg.2003.08.024]

50   **Nagino M**, Nimura Y, Kamiya J, Kondo S, Uesaka K, Kin Y, Kutsuna Y, Hayakawa N, Yamamoto H. Right or left trisegment portal vein embolization before hepatic trisegmentectomy for hilar bile duct carcinoma. *Surgery* 1995; **117**: 677-681 [PMID: 7778031 DOI: 10.1016/S0039-6060(95)80012-3]

51   **Corrêa D**, Schwartz L, Jarnagin WR, Tuorto S, DeMatteo R, D'Angelica M, Allen P, Brown K, Fong Y. Kinetics of liver volume changes in the first year after portal vein embolization. *Arch Surg* 2010; **145**: 351-354; discussion 354-355 [PMID: 20404285 DOI: 10.1001/archsurg.2010.42]

52   **Ribero D**, Abdalla EK, Madoff DC, Donadon M, Loyer EM, Vauthey JN. Portal vein embolization before major hepatectomy and its effects on regeneration, resectability and outcome. *Br J Surg* 2007; **94**: 1386-1394 [PMID: 17583900 DOI: 10.1002/bjs.5836]

53   **Abdalla EK**, Barnett CC, Doherty D, Curley SA, Vauthey JN. Extended hepatectomy in patients with hepatobiliary malignancies with and without preoperative portal vein embolization. *Arch Surg* 2002; **137**: 675-680; discussion 680-681 [PMID: 12049538 DOI: 10.1001/archsurg.137.6.675]

54   **Nagino M**, Nimura Y, Kamiya J, Kondo S, Uesaka K, Kin Y, Hayakawa N, Yamamoto H. Changes in hepatic lobe volume in biliary tract cancer patients after right portal vein embolization. *Hepatology* 1995; **21**: 434-439 [PMID: 7843717 DOI: 10.1002/hep.1840210226]

55   **Imamura H**, Shimada R, Kubota M, Matsuyama Y, Nakayama A, Miyagawa S, Makuuchi M, Kawasaki S. Preoperative portal vein embolization: an audit of 84 patients. *Hepatology* 1999; **29**: 1099-1105 [PMID: 10094953 DOI: 10.1002/hep.510290415]

56   **Farges O**, Belghiti J, Kianmanesh R, Regimbeau JM, Santoro R, Vilgrain V, Denys A, Sauvanet A. Portal vein embolization before right hepatectomy: prospective clinical trial. *Ann Surg* 2003; **237**: 208-217 [PMID: 12560779 DOI: 10.1097/01.SLA.00-00048447.16651.7B]

57   **Aoki T**, Imamura H, Hasegawa K, Matsukura A, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Sequential preoperative arterial and portal venous embolizations in patients with hepatocellular carcinoma. *Arch Surg* 2004; **139**: 766-774 [PMID: 15249411 DOI: 10.1001/archsurg.139.7.766]

58   **Ogata S**, Belghiti J, Farges O, Varma D, Sibert A, Vilgrain V. Sequential arterial and portal vein embolizations before right hepatectomy in patients with cirrhosis and hepatocellular carcinoma. *Br J Surg* 2006; **93**: 1091-1098 [PMID: 16779884 DOI: 10.1002/bjs.5341]

59   **Seo DD**, Lee HC, Jang MK, Min HJ, Kim KM, Lim YS, Chung YH, Lee YS, Suh DJ, Ko GY, Lee YJ, Lee SG. Preoperative portal vein embolization and surgical resection in patients with hepatocellular carcinoma and small future liver remnant volume: comparison with transarterial chemoembolization. *Ann Surg Oncol* 2007; **14**: 3501-3509 [PMID: 17899289 DOI: 10.1245/s10434-007-9553-y]

60   **Azoulay D**, Castaing D, Krissat J, Smail A, Hargreaves GM, Lemoine A, Emile JF, Bismuth H. Percutaneous portal vein embolization increases the feasibility and safety of major liver resection for hepatocellular carcinoma in injured liver. *Ann Surg* 2000; **232**: 665-672 [PMID: 11066138 DOI: 10.1097/00000658-200011000-00008]

61   **Tanaka H**, Hirohashi K, Kubo S, Shuto T, Higaki I, Kinoshita H. Preoperative portal vein embolization improves prognosis after right hepatectomy for hepatocellular carcinoma in patients with impaired hepatic function. *Br J Surg* 2000; **87**: 879-882 [PMID: 10931022 DOI: 10.1046/j.1365-2168.2000.01438.x]

62   **Wakabayashi H**, Ishimura K, Okano K, Izuishi K, Karasawa Y, Goda F, Maeba T, Maeta H. Is preoperative portal vein embolization effective in improving prognosis after major hepatic resection in patients with advanced-stage hepatocellular carcinoma? *Cancer* 2001; **92**: 2384-2390 [PMID: 11745294 DOI: 10.1002/1097-0142(20011101)]

63   **Hayashi S**, Baba Y, Ueno K, Nakajo M, Kubo F, Ueno S, Aikou

T, Komokata T, Nakamura N, Sakata R. Acceleration of primary liver tumor growth rate in embolized hepatic lobe after portal vein embolization. *Acta Radiol* 2007; **48**: 721-727 [PMID: 17729001 DOI: 10.1080/02841850701424514]

64   **Nagino M**, Nimura Y, Kamiya J, Kondo S, Kanai M. Selective percutaneous transhepatic embolization of the portal vein in preparation for extensive liver resection: the ipsilateral approach. *Radiology* 1996; **200**: 559-563 [PMID: 8685357 DOI: 10.1148/radiology.200.2.8685357]

65   **Gibo M**, Unten S, Yogi A, Nakayama T, Ayukawa Y, Gibo S, Murayama S, Takara M, Shiraishi M. Percutaneous ipsilateral portal vein embolization using a modified four-lumen balloon catheter with fibrin glue: initial clinical experience. *Radiat Med* 2007; **25**: 164-172 [PMID: 17514367 DOI: 10.1007/s11604-007-0120-z]

66   **Madoff DC**, Abdalla EK, Gupta S, Wu TT, Morris JS, Denys A, Wallace MJ, Morello FA, Ahrar K, Murthy R, Lunagomez S, Hicks ME, Vauthey JN. Transhepatic ipsilateral right portal vein embolization extended to segment IV: improving hypertrophy and resection outcomes with spherical particles and coils. *J Vasc Interv Radiol* 2005; **16**: 215-225 [PMID: 15713922 DOI: 10.1097/01.RVI.0000147067.79223.85]

67   **Shaw CM**, Madoff DC. Acute Thrombosis of Left Portal Vein during Right Portal Vein Embolization Extended to Segment 4. *Semin Intervent Radiol* 2011; **28**: 156-161 [PMID: 22654253 DOI: 10.1055/s-0031-1280655]

68   **Nagasue N**, Yukaya H, Ogawa Y, Kohno H, Nakamura T. Human liver regeneration after major hepatic resection. A study of normal liver and livers with chronic hepatitis and cirrhosis. *Ann Surg* 1987; **206**: 30-39 [PMID: 3038039]

69   **Chen MF**, Hwang TL, Hung CF. Human liver regeneration after major hepatectomy. A study of liver volume by computed tomography. *Ann Surg* 1991; **213**: 227-229 [PMID: 1998403 DOI: 10.1097/00000658-199103000-00008]

70   **Yamanaka N**, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]

71   **Nagino M**, Nimura Y, Kamiya J, Kanai M, Hayakawa N, Yamamoto H. Immediate increase in arterial blood flow in embolized hepatic segments after portal vein embolization: CT demonstration. *AJR Am J Roentgenol* 1998; **171**: 1037-1039 [PMID: 9762992 DOI: 10.2214/ajr.171.4.9762992]

72   **Elias D**, De Baere T, Roche A, Mducreux J, Lasser P. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. *Br J Surg* 1999; **86**: 784-788 [PMID: 10383579 DOI: 10.1046/j.1365-2168.1999.01154.x]

73   **Kokudo N**, Tada K, Seki M, Ohta H, Azekura K, Ueno M, Ohta K, Yamaguchi T, Matsubara T, Takahashi T, Nakajima T, Muto T, Ikari T, Yanagisawa A, Kato Y. Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology* 2001; **34**: 267-272 [PMID: 11481611 DOI: 10.1053/jhep.2001.26513]

74   **de Graaf W**, van Lienden KP, van den Esschert JW, Bennink RJ, van Gulik TM. Increase in future remnant liver function after preoperative portal vein embolization. *Br J Surg* 2011; **98**: 825-834 [PMID: 21484773 DOI: 10.1002/bjs.7456]

75   **Okabe H**, Beppu T, Ishiko T, Masuda T, Hayashi H, Otao R, Hasita H, Okabe K, Sugiyama S, Baba H. Preoperative portal vein embolization (PVE) for patients with hepatocellular carcinoma can improve resectability and may improve disease-free survival. *J Surg Oncol* 2011; **104**: 641-646 [PMID: 21520093 DOI: 10.1002/jso.21928]

76   **Xu C**, Lv PH, Huang XE, Wang SX, Sun L, Wang FA, Wang LF. Safety and efficacy of sequential transcatheter arterial chemoembolization and portal vein embolization prior to major hepatectomy for patients with HCC. *Asian Pac J Cancer Prev*

2014; **15**: 703-706 [PMID: 24568482 DOI: 10.7314/APJCP.2014.15.2.703]

77   **Bismuth H**, Adam R, Lévi F, Farabos C, Waechter F, Castaing D, Majno P, Engerran L. Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. *Ann Surg* 1996; **224**: 509-520; discussion 520-522 [PMID: 8857855 DOI: 10.1097/00000658-199610000-00009]

78   **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 DOI: 10.1097/00000658-200012000-00006]

79   **Moussa ME**, Bean AG, Habib NA. Repeated resection for malignant liver tumours. *Ann R Coll Surg Engl* 1995; **77**: 364-368 [PMID: 7486764]

80   **de Jong MC**, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, Choti MA, Aldrighetti L, Capussotti L, Pawlik TM. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Ann Surg* 2009; **250**: 440-448 [PMID: 19730175 DOI: 10.1097/SLA.0b013e3181b4539b]

81   **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]

82   **Jamal MH**, Hassanain M, Chaudhury P, Tran TT, Wong S, Yousef Y, Jozaghi Y, Salman A, Jabbour S, Simoneau E, Al-Abbad S, Al-Jiffry M, Arena G, Kavan P, Metrakos P. Staged hepatectomy for bilobar colorectal hepatic metastases. *HPB* (Oxford) 2012; **14**: 782-789 [PMID: 23043668 DOI: 10.1111/j.1477-2574.2012.00543.x]

83   **Tsai S**, Marques HP, de Jong MC, Mira P, Ribeiro V, Choti MA, Schulick RD, Barroso E, Pawlik TM. Two-stage strategy for patients with extensive bilateral colorectal liver metastases. *HPB* (Oxford) 2010; **12**: 262-269 [PMID: 20590896 DOI: 10.1111/j.1477-2574.2010.00161.x]

84   **Togo S**, Nagano Y, Masui H, Tanaka K, Miura Y, Morioka D, Endo I, Sekido H, Ike H, Shimada H. Two-stage hepatectomy for multiple bilobular liver metastases from colorectal cancer. *Hepatogastroenterology* 2005; **52**: 913-919 [PMID: 15966231]

85   **Shimada H**, Tanaka K, Masui H, Nagano Y, Matsuo K, Kijima M, Ichikawa Y, Ike H, Ooki S, Togo S. Results of surgical treatment for multiple (> or =5 nodules) bi-lobar hepatic metastases from colorectal cancer. *Langenbecks Arch Surg* 2004; **389**: 114-121 [PMID: 14714186 DOI: 10.1007/s00423-003-0447-6]

86   **Aussilhou B**, Lesurtel M, Sauvanet A, Farges O, Dokmak S, Goasguen N, Sibert A, Vilgrain V, Belghiti J. Right portal vein ligation is as efficient as portal vein embolization to induce hypertrophy of the left liver remnant. *J Gastrointest Surg* 2008; **12**: 297-303 [PMID: 18060468 DOI: 10.1007/s11605-007-0410-x]

87   **Heinrich S**, Jochum W, Graf R, Clavien PA. Portal vein ligation and partial hepatectomy differentially influence growth of intrahepatic metastasis and liver regeneration in mice. *J Hepatol* 2006; **45**: 35-42 [PMID: 16698111 DOI: 10.1016/j.jhep.2006.02.020]

88   **Baumgart JLS**, Lang H. A new method for induction of liver hypertrophy prior to right trisectionectomy: a report of three cases. *HPB* (Oxford) 2011; (**13**): 71-72 [DOI: 10.1111/j.1477-2574.2011.00308.x]

89   **de Santibañes E**, Clavien PA. Playing Play-Doh to prevent postoperative liver failure: the "ALPPS" approach. *Ann Surg* 2012; **255**: 415-417 [PMID: 22330039 DOI: 10.1097/SLA.0b013e318248577d]

90   **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]

91   **Cavaness KM**, Doyle MB, Lin Y, Maynard E, Chapman WC. Using ALPPS to induce rapid liver hypertrophy in a patient with hepatic fibrosis and portal vein thrombosis. *J Gastrointest Surg* 2013; **17**: 207-212 [PMID: 22996934 DOI: 10.1007/s11605-012-2029-9]

92   **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]

93   **Vennarecci G**, Laurenzi A, Levi Sandri GB, Busi Rizzi E, Cristofaro M, Montalbano M, Piselli P, Andreoli A, D'Offizi G, Ettorre GM. The ALPPS procedure for hepatocellular carcinoma. *Eur J Surg Oncol* 2014; **40**: 982-988 [PMID: 24767805 DOI: 10.1016/j.ejso.2014.04.002]

94   **Levi Sandri GB**, Lai Q, Rayar M, Sulpice L. ALPPS procedure for hepatocellular carcinoma with macrovascular thrombosis: a new opportunity? *J Hepatol* 2015; **62**: 241-242 [PMID: 25200909 DOI: 10.1016/j.jhep.2014.08.048]

95   **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]

96   **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]

97   **Ielpo B**, Quijano Y, Vicente E. Pearls and pitfalls on ALPPS procedure: new complications in a new technique. *Updates Surg* 2014; **66**: 159-161 [PMID: 24584837 DOI: 10.1007/s13304-014-0249-0]

98   **Chan AC**, Pang R, Poon RT. Simplifying the ALPPS procedure by the anterior approach. *Ann Surg* 2014; **260**: e3 [PMID: 24866543 DOI: 10.1097/SLA.0000000000000736]

99   **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]

100   **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]

101   **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]

MINIREVIEWS

Advances in hepatocellular carcinoma: Nonalcoholic steatohepatitis-related hepatocellular carcinoma

Fauzia Z Khan, Ryan B Perumpail, Robert J Wong, Aijaz Ahmed

Fauzia Z Khan, Ryan B Perumpail, Aijaz Ahmed, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA 94305, United States

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

Author contributions: Khan FZ, Perumpail RB, Wong RJ and Ahmed A developed an outline for the review article; Khan FZ, Perumpail RB, Wong RJ and Ahmed A performed research; Khan FZ, Perumpail RB, Wong RJ and Ahmed A performed a medline search to review the published data on this topic; Khan FZ and Perumpail RB prepared the initial and final draft based on input from senior authors; Wong RJ and Ahmed A reviewed each section of the manuscript and provided critical mentorship to complete this review article.

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

Correspondence to: Aijaz Ahmed, MD, Associate Professor of Medicine, Medical Director Liver Transplant Program, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 750 Welch Road, Suite 210, Palo Alto, Stanford, CA 94305, United States. aijazahmed@stanford.edu
Telephone: +1-650-4986091
Fax: +1-650-4985692

Published online: August 28, 2015

Abstract

An increase in the prevalence of obesity and diabetes mellitus has been associated with the rise in non-alcoholic fatty liver disease (NAFLD). Two-thirds of the obese and diabetic populations are estimated to develop NAFLD. Currently, NAFLD is the most common etiology for chronic liver disease globally. The clinical spectrum of NAFLD ranges from simple steatosis, an accumulation of fat greater than 5% of liver weight, to nonalcoholic steatohepatitis (NASH), a more aggressive form with necroinflammation and fibrosis. Among the patients who develop NASH, up to 20% may advance to cirrhosis and are at risk for complications of end-stage liver disease. One of the major complications observed in patients with NASH-related cirrhosis is hepatocellular carcinoma (HCC), which has emerged as the sixth most common cancer and second leading etiology of cancer-related deaths worldwide. The incidence of HCC in the United States alone has tripled over the last three decades. In addition, emerging data are suggesting that a small proportion of patients with NAFLD may be at higher risk for HCC in the absence of cirrhosis - implicating obesity and diabetes mellitus as potential risk factors for HCC.

Key words: Hepatocellular carcinoma; Nonalcoholic fatty liver disease; Obesity; Insulin resistance; Nonalcoholic steatohepatitis; Overweight

Core tip: The worldwide rise in overweight and obesity has been associated with increasing rates of nonalcoholic fatty liver disease (NAFLD), which is now the most common etiology of chronic liver disease. The more aggressive form of NAFLD, nonalcoholic steatohepatitis (NASH), promotes the development

of hepatocellular carcinoma (HCC). As NASH-related cirrhosis has emerged as the most rapidly increasing indication for HCC-related liver transplantation in the United States, new strategies for HCC surveillance and targeted therapies in this patient population are warranted.

INTRODUCTION

Increases in the prevalence of obesity and diabetes mellitus (DM) have been associated with the rise in nonalcoholic fatty liver disease (NAFLD). Currently, NAFLD is the most common cause of chronic liver disease worldwide. The clinical spectrum of NAFLD ranges from simple steatosis, an accumulation of fat greater than 5% of liver weight, to nonalcoholic steatohepatitis (NASH), a more aggressive form with inflammation and necrosis. NAFLD afflicts an estimated 30%-40% of the adult population in the United States. Even though the majority of these patients remain stable, up to 25% of patients with NAFLD can progress to NASH. Among the patients who develop NASH, many advance to cirrhosis and are at risk for complications of end-stage liver disease[1-4]. One of the major complications observed in patients with NASH-related cirrhosis is hepatocellular carcinoma (HCC), which has emerged as the sixth most common cancer and second leading etiology of cancer-related deaths worldwide[5].

NASH AND ITS ASSOCIATION WITH HCC

The incidence of HCC in the United States alone has tripled over the last three decades. A recent study evaluated the trends in the age-adjusted incidence rates of HCC utilizing the Surveillance, Epidemiology, and End Result (SEER) database. It revealed a rise in the incidence rate of HCC from 1.6 per 100000 in 1975 to 4.9 per 100000 in 2000[6]. A notable increase was observed among Hispanic, Black and White males (Figures 1 and 2). Historically, the leading etiologies underlying HCC have included hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease and other chronic liver diseases[7]. In the majority of cases, HCC develops in the setting of cirrhosis with the exception of HBV. A significant number of patients with HBV can develop HCC in the absence of cirrhosis because HBV itself is a known carcinogen. Emerging data are suggesting that a small proportion of patients with NAFLD may be at higher risk for HCC in the absence of

cirrhosis - implicating obesity as an independent risk factor for HCC[7-10]. Globally, HBV-related liver disease is the leading etiology underlying HCC. In the United States, the increase in the incidence of HCC is mainly attributable to chronic HCV infection, particularly as the baby boomer generation ages. However, the etiology in a significant proportion of cases remains unclear, indicating that other risk factors play an important role as well. Nevertheless, as NAFLD has become the leading cause of chronic liver disease in developing and developed countries plagued by rising rates of obesity, DM and the metabolic syndrome, the prevalence and impact of NAFLD is expected to rise and drive the epidemic of HCC in the United States Two-thirds of those who suffer from obesity and DM are estimated to suffer from NAFLD as well[8,9]. NASH has a prevalence of 2%-5% in the United States, with up to 20% of patients exhibiting cirrhosis[10]. Similar to HCV patients with cirrhosis, patients with NASH-related cirrhosis are also at an increased risk of developing HCC. In a single-center study, Ascha *et al*[11] compared the incidence of HCV and NASH-related cirrhosis. Among 510 patients with cirrhosis, 195 had underlying NASH, while 315 had cirrhosis secondary to HCV. Median follow-up of 3.2 years revealed an annual cumulative HCC incidence of 2.6% for NASH-related cirrhosis as compared to 4% for HCV-related cirrhosis cases. Additional large population-based studies with longer durations of follow-up are needed to re-confirm that patients with NASH-related cirrhosis carry a significant risk of developing HCC and should be closely monitored.

Despite the estimated low HCC incidence rate of 2.6% in patients with NASH-related cirrhosis, the surge in the number of cases with NAFLD is projected to lead to an increase in the number of patients with NASH-related HCC. A recent study by Wong *et al*[12] demonstrated a nearly fourfold increase in the prevalence of NASH-related HCC cases among liver transplant recipients since implementation of the model for end-stage liver disease in 2002[12]. In a large United States population-based study utilizing the United Network for Organ Sharing database from 2002-2012, Wong *et al*[12] reported 10061 patients with HCC among 61868 liver transplant recipients. In order to achieve a more accurate assessment of the true prevalence of NASH, Wong *et al*[12] created a modified NASH category, which included patients with a formal diagnosis of NASH as well as obese patients [body mass index (BMI) > 30 kg/m^2] with cryptogenic cirrhosis and obese patients with unknown etiology of HCC. The proportion of HCC patients undergoing liver transplantation increased from 3.3% in the year 2000 to 23.3% in 2012 (Table 1). Although HCV remained the leading etiology of HCC, NASH was found to be the second leading cause of HCC in patients undergoing liver transplantation and the most rapidly growing indication for liver transplantation among patients with HCC in the United States. Yet, despite increased rates of liver transplantation among patients with NASH, these patients have poorer liver
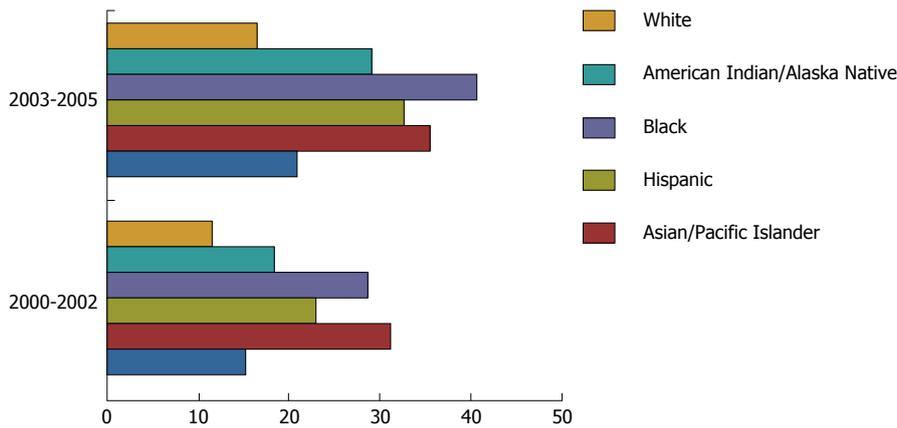
Figure 1 Hepatocellular carcinoma incidence rates (per 100000) in males in the United States, age 50-59 years by race/ethnicity from 2000-2002 and 2003-2005[6]. The dark blue bars on the bottom of each panel indicate the overall incidence rates in men age 50-59 years during that time period.
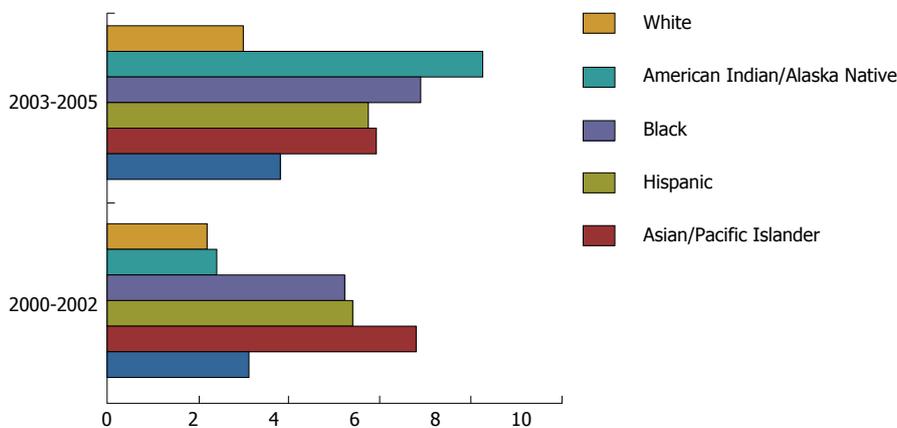
Figure 2 Hepatocellular carcinoma incidence rates (per 100000) in females in the United States, age 50-59 years by race/ethnicity from 2000-2002 and 2003-2005[6]. The dark blue bars on the bottom of each panel indicates the overall incidence rates in women age 50-59 years during that time period.

Table 1 The etiologies of liver disease among hepatocellular carcinoma related liver transplant recipients following the implementation of model for end-stage liver disease system in 2002 for prioritizing of patients for liver transplant[12]

Etiologies of HCC	HCC patients undergoing liver transplantation in the MELD era		
	2002	2007	2012
HCV	43.4%	46.3%	49.9%
NASH	0%	4.0%	6.0%
ALD + HCV	4.9%	6.5%	6.4%
HBV	10.2%	8.3%	4.6%
Modified NASH	8.3%	10.3%	13.5%

HCC: Hepatocellular carcinoma; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NASH: Nonalcoholic steatohepatitis.

transplant waitlist survival and are less likely to undergo liver transplantation than patients with HCV or alcoholic liver disease[13].

RISK FACTORS FOR NASH-RELATED HCC

The remarkable increase in the number of HCC cases in developed countries is linked to several important risk factors. Half of the new cases of HCC are attributable to HCV, whereas the etiology of cirrhosis leading to HCC in 15%-50% of new cases remains unclear[1,6]. With the growing burden of obesity and DM in developed countries leading to NAFLD and NASH, there is cumulative evidence suggesting that NASH may account for a large proportion of these cases of idiopathic or cryptogenic cirrhosis[1,14]. NASH-related HCC patients undergoing liver transplantation have significantly higher rates of DM and higher BMI[12]. Similar to the NASH population, patients with cryptogenic cirrhosis have a high prevalence of obesity and DM as well. Additionally, a significant number of liver transplant recipients with cryptogenic cirrhosis develop NAFLD (25.4%) or NASH (15.7%) within 2 years following transplant surgery[15]. This provides further support that patients with end stage or burned out NASH are potentially being misclassified with cryptogenic cirrhosis. Typically, in patients with NASH-related cirrhosis characteristic histologic features of NASH, including steatosis, lobular inflammation, balloon degeneration, necrosis and Mallory bodies in zone 3 are not evident, and may lead to misdiagnosis.

Regardless of the underlying cause of liver disease, cirrhosis is believed to be a major risk factor for the development of HCC. Yasui *et al*[16] performed a multi-

center retrospective study in Japan to understand the pathological course of NASH progressing to HCC. These investigators identified 19 patients with histologically proven NASH who developed HCC during a median follow-up period of 3.8 years. Patients were screened and underwent surveillance using ultrasound, computed tomography, des-gamma-carboxyprothrombin testing, and AFP testing. The majority of the patients were elderly, obese (84%), diabetic (58%), and hypertensive (63%). When histopathology at the time of NASH diagnosis was compared to histopathology at the time of HCC diagnosis, the degree of steatosis remained unchanged in the majority of patients (86%), but the stage of fibrosis was significantly more advanced at the time of HCC diagnosis ($P = 0.02$). These findings suggest that fibrosis is a significant risk factor for the development of HCC among patients with NASH. However, there were 3 male patients (21%) of 19 who only had stage 2 fibrosis at the time of HCC diagnosis. Interestingly, in an earlier cross-sectional study performed by Yasui *et al*[16], 28% of NASH patients, with an older male predominance, only had stage 1 or stage 2 fibrosis at the time of HCC diagnosis. These findings indicate that HCC can occur in NASH patients regardless of the degree of fibrosis, particularly in older men. Although the association between cirrhosis secondary to NAFLD and HCC is well documented, few recent studies have pointed toward a positive correlation between non-cirrhotic fatty liver disease and HCC. The prevalence of histologically confirmed steatosis and steatohepatitis is about 54% and 15% in HCC cases without any underlying cirrhosis, respectively[17]. In another retrospective study, cirrhosis was detected in only 53% of NASH-related HCC patients, which was significantly less than in the group with non-NASH HCC (90%). Therefore, though cirrhosis is a crucial risk factor, NASH is likely a significant independent risk factor for HCC[18]. Future prospective, large population-based studies with longer durations of follow-up are needed to determine the validity of this potentially important observation.

NAFLD is considered the hepatic manifestation of the metabolic syndrome, which includes a cluster of interlinked metabolic risk factors, such as hypertension, hyperglycemia, central obesity and dyslipidemia. The majority of patients with HCC secondary to NAFLD have two or more types of metabolic disease, the most common being hyperglycemia due to insulin resistance. Based on a large United States population-based study using the SEER-Medicare database of patients over 65 years of age with histologically diagnosed HCC between 1994 and 2005, Welzel *et al*[19] concluded that the metabolic syndrome is an important risk factor for HCC development. Comparison with a randomly selected control group revealed that each component of the metabolic syndrome was individually associated with a statistically significant increased risk of HCC development ($P < 0.0001$).

Obesity is considered a significant risk factor for the development of not only HCC but also for a number of other malignancies. Welzel *et al*[19] reported a 1.93-fold increased risk of HCC in the obese population. In a large prospective study by Calle *et al*[20], out of 900000 United States adults who were enrolled in the study in 1982, 57145 died from cancer during the 16 year follow-up period. Morbidly obese (BMI > 40 kg/m^2) males had a 52% higher mortality, while morbidly obese women had a 62% higher mortality compared to corresponding mortality in their normal weight counterparts. There is growing evidence supporting a positive correlation between excess body weight and HCC risk. Results from a meta-analysis of 11 cohort studies performed in 2007 showed that the liver cancer risk was 17% among the overweight and 89% among the obese as compared to those with normal weight. Furthermore, it was estimated that 28% of liver cancer cases in men and 27% of liver cancer cases in women were linked to overweight or obesity (BMI > 25 kg/m^2)[21]. Even among those with normal weight, visceral fat accumulation is considered pro-oncogenic and is a potential risk factor for the recurrence of HCC. Ohki *et al*[22] investigated whether visceral adiposity increased the risk of cancer recurrence in patients with NASH-related HCC after undergoing curative treatment with radiofrequency ablation. Those with higher visceral fat area (> 130 cm^2 in males, > 90 cm^2 in females) were at increased risk of recurrent HCC (75%), compared to those with lower visceral fat area (45%).

DM, another important risk factor for HCC, is associated with 2.90 fold increased risk of HCC[19]. Insulin resistance in patients with DM leads to the increased release of free fatty acids and pro-inflammatory cytokines, including tumor necrosis factor (TNF) alpha, leptin, and interleukin-6 (IL-6), as well as reactive oxygen species, which favor fat deposition and inflammation in the liver[1,20]. Statins have been reported to have a cancer preventative effect if prescribed to patients with DM[23]. Among a large cohort of patients diagnosed with DM, a matched, nested case control study of 1303 diabetics with HCC and 5212 diabetics without HCC demonstrated that increased duration and frequency of statin prescriptions in diabetics was associated with a decreased incidence of HCC (risk reduction: 25%-40%). However, the study was limited by its retrospective design and relatively short length of follow-up (median 2.4 years). Further studies exploring this anti-inflammatory role of statins and their potentially beneficial role in preventing HCC development may have significant clinical implications.

Hepatic iron overload is also being increasingly recognized as a clinically important risk factor contributing to the development of NASH and its progression to HCC[24]. In patients with hereditary hemochromatosis, excess iron deposits drive oxidative stress, leading to the production of iron catalyzed oxyradicals. These oxyradicals contribute to the progression of fibrosis to cirrhosis and later to HCC. Lesser degrees of iron accumulation are frequently observed in patients with other forms of liver diseases, such as alcoholic liver
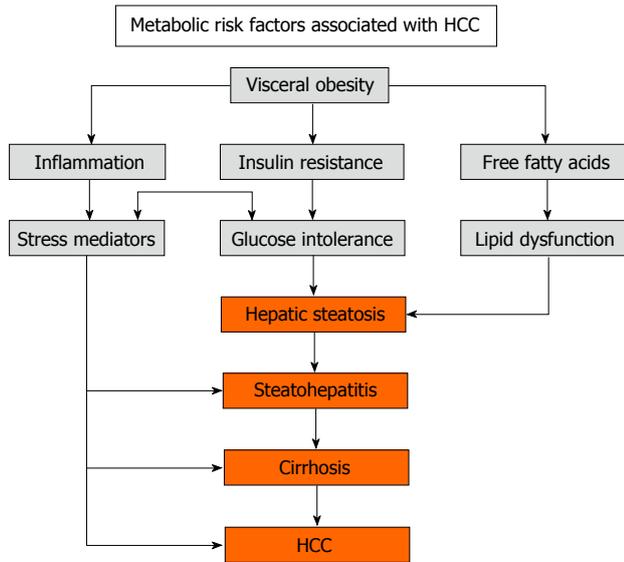
Figure 3 Metabolic pathogenetic pathways to hepatocellular carcinoma. HCC: Hepatocellular carcinoma.

disease, HCV-related liver cirrhosis and NAFLD. A study by Sorrentino *et al*[24] retrospectively assessed the hepatic iron stores in 51 patients with HCC diagnosis and NASH-related cirrhosis and 102 HCC-free controls with NASH-related cirrhosis. Conditional regression analysis revealed that histologic sinusoidal iron deposition was larger in size and more frequent in HCC patients than in controls.

In the aforementioned study by Ascha *et al*[11] that evaluated the annual cumulative incidence of HCC in patients with NASH-related cirrhosis, the investigators also studied and identified additional risk factors impacting HCC development in these patients. Old age (P = 0.006) and any lifetime alcohol consumption (P = 0.002) were statistically significant risk factors for progression to NASH-related cirrhosis and development of HCC. While heavy consumption of alcoholic beverages is widely considered a major risk factor for development of HCC, the study highlights that any lifetime alcohol consumption among patients with NASH was associated with a 3.6 times increased risk of HCC compared to those without lifetime alcohol consumption (P = 0.003). This suggests that alcohol consumption is a modifiable risk factor; even in quantities generally considered safe may increase the risk of HCC development in patients with NASH-related cirrhosis.

MECHANISMS UNDERLYING NASH-RELATED HCC

Numerous unique mechanisms underlie the pathogenesis of NASH-related HCC (Figure 3). Insulin resistance, associated with NAFLD, predisposes to the production of free fatty acids and several pro-inflammatory cytokines, including TNF-α and IL-6[1]. TNF-α promotes pro-oncogenic pathways, which specifically involve nuclear factor κB, c-Jun amino acid-terminal kinase (JNK), and

mammalian target of rapamycin complex (mTOR)[25,26]. Obesity is associated with increased IL-6 levels, while weight loss reduces levels of TNF-α and IL-6, resulting in a decreased inflammatory and potentially carcinogenic response[27]. Prolonged upregulation of the IL-6/STAT3 axis results in an increased probability that hepatocytes that have already acquired oncogenic mutations from exposure to carcinogens will continue malignant transformation[28]. Insulin resistance upregulates the production of insulin-like growth factor-1 (IGF-1). IGF-1 promotes processes linked to HCC development, such as expression of proto-oncogenes c-fos and c-jun *in vitro* and activating mitogen activated protein kinases (MAPK)[29]. JNK, a MAPK, is activated by IR and down-regulated by weight loss[30]. Histopathological analysis reveals that 70% of HCC tissue specimens stain positive for phosphorylated JNK, suggesting its role in the development of HCC[31]. Overall, several mechanisms underlying NASH-related HCC have been elucidated and pave the way for new therapeutic targets.

SURVEILLANCE OF HCC

The majority of cases of HCC occur in patients with known cirrhosis. United States and European societies recommend regular surveillance of HCC in cirrhotic patients on a semiannual basis[32,33]. Ultrasound is regarded as the most effective tool for regular surveillance, while the role of AFP as a reliable tool for surveillance is still controversial due to high false positive and negative rates[34]. Despite evidence of improved survival among patients who underwent regular surveillance for HCC[35], Davila *et al*[36] suggests that the actual implementation of HCC surveillance in the cirrhotic patient population is inadequate. Davila *et al*[36] utilized Medicare databases to identify 1873 HCC patients with underlying cirrhosis between 1994 and 2002 and noted that among these patients only 17% received regular surveillance, while 38% received inconsistent surveillance prior to the diagnosis of HCC. Moreover, among all patients who received regular surveillance, 52% had ultrasound and AFP levels, 2% received ultrasound only, and 46% were monitored with AFP level only. Disparities were noted in surveillance, with affluent patients and patients with higher education at increased likelihood of receiving regular surveillance. Future studies would be helpful to explore whether on-time HCC surveillance in NASH patients would be effective in decreasing morbidity and mortality from the disease.

CONCLUSION

Despite several gaps in our current knowledge of NAFLD and NASH, the increasing prevalence of NASH is partly responsible for the current growth in HCC incidence. Ascha *et al*[11] has reported HCC incidence of 2.6% per year in patients with NASH-related cirrhosis. Currently, NASH-related cirrhosis is the most rapidly increasing indication for HCC-related liver transplantation in the

United States[12]. It is essential to control the growing epidemic of obesity and DM. The role of statins in reducing the risk of HCC in patients with DM is an interesting finding which needs to be further evaluated. Timely detection of HCC is difficult due to its indolent clinical course in the initial stages and high index of clinical suspicion is crucial to early diagnosis. Patients with NASH often present at an increased age and after having developed advanced complications of end-stage liver disease, which include inoperable HCC. Efforts to devise noninvasive methods of detecting NASH and maximizing the management of common risk factors shared by NASH and HCC can lead to a potential reduction in liver disease and liver cancer burden.

REFERENCES

1   **Starley BQ**, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820-1832 [PMID: 20432259 DOI: 10.1002/hep.23594]
2   **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]
3   **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]
4   **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]
5   **Ferlay JSI**, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013
6   **Altekruse SF**, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**: 1485-1491 [PMID: 19224838 DOI: 10.1200/JCO.2008.20.7753]
7   **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
8   **Targher G**, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212-1218 [PMID: 17277038 DOI: 10.2337/dc06-2247]
9   **Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]
10   **McCullough AJ**. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 521-533, viii [PMID: 15331061 DOI: 10.1016/j.cld.2004.04.004]
11   **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]
12   **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711]
13   **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]
14   **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]
15   **Ong J**, Younossi ZM, Reddy V, Price LL, Gramlich T, Mayes J, Boparai N. Cryptogenic cirrhosis and posttransplantation nonalcoholic fatty liver disease. *Liver Transpl* 2001; **7**: 797-801 [PMID: 11552214 DOI: 10.1053/jlts.2001.24644]
16   **Yasui K**, Hashimoto E, Tokushige K, Koike K, Shima T, Kanbara Y, Saibara T, Uto H, Takami S, Kawanaka M, Komorizono Y, Okanoue T. Clinical and pathological progression of non-alcoholic steatohepatitis to hepatocellular carcinoma. *Hepatol Res* 2012; **42**: 767-773 [PMID: 22487102 DOI: 10.1111/j.1872-034X.2012.00986.x]
17   **Alexander J**, Torbenson M, Wu TT, Yeh MM. Non-alcoholic fatty liver disease contributes to hepatocarcinogenesis in non-cirrhotic liver: a clinical and pathological study. *J Gastroenterol Hepatol* 2013; **28**: 848-854 [PMID: 23302015 DOI: 10.1111/jgh.12116]
18   **Ertle J**, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, Schlaak JF, Gerken G, Syn WK, Canbay A. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011; **128**: 2436-2443 [PMID: 21128245 DOI: 10.1002/ijc.25797]
19   **Welzel TM**, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology* 2011; **54**: 463-471 [PMID: 21538440 DOI: 10.1002/hep.24397]
20   **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]
21   **Larsson SC**, Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. *Br J Cancer* 2007; **97**: 1005-1008 [PMID: 17700568 DOI: 10.1038/sj.bjc.6603932]
22   **Ohki T**, Tateishi R, Shiina S, Goto E, Sato T, Nakagawa H, Masuzaki R, Goto T, Hamamura K, Kanai F, Yoshida H, Kawabe T, Omata M. Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009; **58**: 839-844 [PMID: 19174415 DOI: 10.1136/gut.2008.164053]
23   **El-Serag HB**, Johnson ML, Hachem C, Morgana RO. Statins are associated with a reduced risk of hepatocellular carcinoma in a large cohort of patients with diabetes. *Gastroenterology* 2009; **136**: 1601-1608 [PMID: 19208359 DOI: 10.1053/j.gastro.2009.01.053]
24   **Sorrentino P**, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, Vecchione R. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis. *J Hepatol* 2009; **50**: 351-357 [PMID: 19070395 DOI: 10.1016/j.jhep.2008.09.011]
25   **Stickel F**, Hellerbrand C. Non-alcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: mechanisms and implications. *Gut* 2010; **59**: 1303-1307 [PMID: 20650925 DOI: 10.1136/gut.2009.199661]
26   **Marra F**. Leptin and liver fibrosis: a matter of fat. *Gastroenterology* 2002; **122**: 1529-1532 [PMID: 11984539 DOI: 10.1053/gast.2002.33369]
27   **Bougoulia M**, Triantos A, Koliakos G. Effect of weight loss with or without orlistat treatment on adipocytokines, inflammation, and oxidative markers in obese women. *Hormones* (Athens) 2006; **5**: 259-269 [PMID: 17178701 DOI: 10.14310/horm.2002.11190]
28   **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]

29   **Price JA**, Kovach SJ, Johnson T, Koniaris LG, Cahill PA, Sitzmann JV, McKillop IH. Insulin-like growth factor I is a comitogen for hepatocyte growth factor in a rat model of hepatocellular carcinoma. *Hepatology* 2002; **36**: 1089-1097 [PMID: 12395318 DOI: 10.1053/jhep.2002.36158]

30   **Cho H**, Black SC, Looper D, Shi M, Kelly-Sullivan D, Timofeevski S, Siegel K, Yu XH, McDonnell SR, Chen P, Yie J, Ogilvie KM, Fraser J, Briscoe CP. Pharmacological chara-cterization of a small molecule inhibitor of c-Jun kinase. *Am J Physiol Endocrinol Metab* 2008; **295**: E1142-E1151 [PMID: 18728225 DOI: 10.1152/ajpendo.90298.2008]

31   **Chang Q**, Zhang Y, Beezhold KJ, Bhatia D, Zhao H, Chen J, Castranova V, Shi X, Chen F. Sustained JNK1 activation is associated with altered histone H3 methylations in human liver cancer. *J Hepatol* 2009; **50**: 323-333 [PMID: 19041150 DOI: 10.1016/j.jhep.2008.07.037]

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

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

34   **Forner A**, Reig M, Bruix J. Alpha-fetoprotein for hepatocellular carcinoma diagnosis: the demise of a brilliant star. *Gastroen-terology* 2009; **137**: 26-29 [PMID: 19482098 DOI: 10.1053/j.gastro.2009.05.014]

35   **Chan AC**, Poon RT, Ng KK, Lo CM, Fan ST, Wong J. Changing paradigm in the management of hepatocellular carcinoma improves the survival benefit of early detection by screening. *Ann Surg* 2008; **247**: 666-673 [PMID: 18362630 DOI: 10.1097/SLA.0b013e31816a747a]

36   **Davila JA**, Morgan RO, Richardson PA, Du XL, McGlynn KA, El-Serag HB. Use of surveillance for hepatocellular carcinoma among patients with cirrhosis in the United States. *Hepatology* 2010; **52**: 132-141 [PMID: 20578139 DOI: 10.1002/hep.23615]

MINIREVIEWS

Biliary complications after pediatric liver transplantation: Risk factors, diagnosis and management

Flavia H Feier, Eduardo A da Fonseca, Joao Seda-Neto, Paulo Chapchap

Flavia H Feier, Eduardo A da Fonseca, Joao Seda-Neto, Paulo Chapchap, Hepatology and Liver Transplantation, Hospital Sirio Libanes, 01308050 Sao Paulo, Brazil

Correspondence to: Flavia H Feier, MD, Transplant Surgeon, Hepatology and Liver Transplantation, Hospital Sirio Libanes, Adma Jafet 115, 01308050 Sao Paulo, Brazil. flavia.feier@gmail.com
Telephone: +55-51-81890439
Fax: +55-51-32484036

Abstract

The expanded indications of partial grafts in pediatric liver transplantation have reduced waiting list mortality. However, a higher morbidity is observed, including an increased rate of biliary complications (BCs). Factors such as the type of graft, the preservation methods applied, the donor characteristics, the type of biliary reconstruction, and the number of bile ducts in the liver graft influences the occurrence of these complications. Bile leaks and strictures comprise the majority of post-transplant BCs. Biliary strictures require a high grade of suspicion, and because most children have a bileo-enteric anastomosis, its diagnosis and management rely on percutaneous hepatic cholangiography and percutaneous biliary interventions (PBI). The success rates with PBI range from 70% to 90%. Surgery is reserved for patients who have failed PBI. BCs in children after liver transplantation have a prolonged treatment and are associated with a longer length of stay and higher hospital costs. However, with early diagnosis and aggressive treatment, patient and graft survival are not significantly compromised.

Key words: Outcomes; Live-donor; Infants; Strictures; Bile leaks

Core tip: Biliary complications in children after liver transplantation cause significant morbidity, which affect quality of life, increase hospital costs and jeopardize the liver graft if they are not treated appropriately. Diagnostic and treatment approaches to the different types of complications are highlighted, as are the technical nuances specific to pediatric recipients.

INTRODUCTION

Pediatric liver transplantation has expanded over the

last several decades due to the utilization of partial grafts, including split and reduced grafts from deceased donors and live-donor grafts. Comparable patient and graft survival to whole liver recipients has been achieved in most centers[1]; however, the reduction in the waiting-list mortality came at a price in regard to post-transplant morbidity, which is mainly represented by the higher incidence of biliary complications (BCs) in recipients of these grafts[1].

BCs are classified into biliary strictures (BS), which can be anastomotic or intrahepatic, bile leaks (BL), bilomas, excluded ducts, stones and cast formation, among others. The overall BC incidence in transplanted children varies from 15% to 40%[2,3]. The type of graft, number of bile ducts and type of biliary reconstruction performed influence the variability in the incidence of BC. The diagnosis, especially in cases of BS, requires a high index of suspicion, and only with early diagnosis and prompt management can high success rates be expected[4]. In children, due to underlying liver disease, the majority of the biliary reconstruction is a bilioenteric (BE) anastomosis performed by a Roux-en-Y. The preferred method to access the biliary anastomosis in cases of BC is using a percutaneous transhepatic cholangiography (PTC) to perform the percutaneous biliary intervention (PBI). PTC and PBI have become the mainstay of diagnosis and treatment in liver transplanted children, with success rates between 70% and 90%[4-6].

CLINICAL PRESENTATION AND DIAGNOSIS

The presentation of BC is quite variable. BL usually have a straightforward diagnosis and present early in the post-transplant course. BS has a more indolent evolution and presents later in the post-transplant follow-up. BS demands a high index of suspicion because in the initial phases the clinical picture can be confused with rejection, infection and primary disease recurrence. Early complications (< 30 d post-transplant) are a result of technical issues such as handling and harvesting of the graft, preservation injuries, surgical technique of biliary reconstruction, or even vascular insufficiency[7]. Late complications (> 90 d post-transplant) can appear from anastomotic (AS) and non-anastomotic strictures (NAS). NAS can be related to the use of ABO-incompatible grafts, preservation injury, opportunistic infections, recurrent hepatitis, ductopenic rejection, recurrent primary sclerosing cholangitis (PSC), stones or casts, post-transplant lymphoproliferative disorder or other tumors[7]. Risk factors for AS complications (leaks and strictures) are linked and include a prolonged cold ischemia time, hepatic artery thrombosis (HAT), CMV infection, and chronic rejection. Tissue hypoxia, as occurs in patients with hepatopulmonary syndrome, at level of the anastomosis can increase the rate of BCs following liver transplantation[8]. The presence of multiple bile ducts was found to be an independent risk factor for the development of BC after pediatric liver transplantation and has a higher incidence of BL compared with a single duct (21% *vs* 9%, respectively)[9].

BL

BL can occur at the anastomosis, T-tube insertion, or cut surface of partial liver grafts. Their incidence after pediatric liver transplantation ranges from 2% to 15%[10,11]. They usually present early in the post-operative course and are diagnosed either by bilious secretion in the abdominal drain or by routine cholangiography in those cases where a t-tube or trans-AS stent was used[7,12]. Bilomas result from small self-limited BL that form collections. They result from the inadvertent division of bile radicals during hilar dissection or parenchymal division in partial grafts. They are diagnosed by ultrasound (US) or computed tomography (CT)[2].

AS STRICTURES

An AS stricture is a segmental narrowing around the anastomosis. The incidence of AS in children varies between 2% and 35%[9,12]. The clinical presentation can be poor, but jaundice, acholic stools, fever and alteration of liver enzymes are common findings[4,12]. A high gamma-glutamyl transferase (GGT) is present in patients with BS[4,13], and an increased GGT value can be related to a greater stricture severity[14]. Usually, the diagnostic workup starts with non-invasive imaging studies, including US, cholangio CT and cholangio magnetic resonance (MR)[7]. US sensitivity is highly variable between studies and ranges from 23% to 92%[2,13,15]. In the pediatric population, US showed only mild biliary dilation in 86.6% of the patients who were diagnosed with severe strictures on PTC[14]. Potthoff *et al*[16] compared US with cholangiography in adults who underwent liver transplantation and found that US was able to detect a BC in 52.4% of the patients. The sensitivity of US to detect AS was 24%, with a specificity of 100%. The lack of US sensitivity may occur because early strictures do not cause the bile ducts to dilate immediately. Due to denervation of the implanted liver, it takes up to three months for the dilation to be detected by US[16].

Cholangio MR is routinely performed in adult recipients as part of the diagnostic work up of BS. It was shown that cholangio MR sensitivity and specificity can be as high as 90% for the diagnosis of BS[17]. In cases of BE anastomosis, however, the sensitivity of cholangio MR drops to 50%[18]. This is important for the pediatric population because most pediatric patients have a BE anastomosis. However, cholangio MR is helpful when studying patients with two separate anastomoses, without significant dilation on US, and with clinical suspicion of BS. It can also serve as a guide to a PTC for identifying the exact puncture location[4]. Further studies

Table 1 Liver biopsy findings suggestive of biliary strictures and differential diagnosis (BANFF[18])			
Histopathological features	**PSC/BS**	**Chronic rejection**	**Primary biliary cirrhosis**
Distribution, severity and composition of portal inflammation	Usually patchy to diffuse; mild neutrophilic, eosinophilic, or occasionally mononuclear predominant	Patchy; usually minimal or mild lymphoplasmacytic	Noticeably patchy and variable intensity; predominantly mononuclear; nodular aggregates and granulomas
Presence and type of interface activity	Prominent and defining feature: ductular type with portal and periportal edema	Minimal to absent	Important feature later in disease development: ductular and necroinflammatory-type with copper deposition
Bile duct inflammation and damage	Periductal lamellar edema "fibrous cholangitis"; acute cholangitis; multiple intra-portal ductal profiles	Focal ongoing lymphocytic bile duct damage; inflammation wanes with duct loss	Granulomatous or focally severe lymphocytic cholangitis is diagnostic in proper setting
Biliary epithelial senescence changes and small bile duct loss	Small bile duct loss associated with ductular reaction	Senescence/atrophy/atypia involve a majority of remaining ducts	Small bile duct loss associated with ductular reaction
Perivenular mononuclear inflammation and/or hepatocyte dropout	Absent	Usually present but variable	Variable but generally mild; if present, involves a minority of perivenular regions
Lobular findings and necroinflammatory activity	Disarray unusual; neutrophil clusters; ± cholestasis	Variable; if present, concentrated in perivenular regions	Mild disarray; parenchymal granulomas; periportal copper deposition and cholestasis are late features
Pattern of fibrosis during progression towards cirrhosis	Biliary pattern	Uncommon; if present usually a venocentric pattern; may evolve to biliary pattern	Biliary pattern

PSC: Primary sclerosing cholangitis; BS: Biliary strictures.

Figure 1 An 18-mo-old girl presented with a severe bilioenteric anastomotic stricture five months after receiving a left lateral segment graft from a live-donor. After two percutaneous biliary interventions failed attempts to cross the stricture the patient underwent surgical revision and a new bilioenteric anastomosis was fashioned.

in children are necessary to define the diagnostic yield of cholangio MR for BS.

Liver biopsy can help elucidate the investigation and provide a differential diagnosis[19] (Table 1). Histology consistent with cholestasis due to biliary obstruction has been found in 69%-83% of the patients with BS[6,13].

The gold standard for the diagnosis and treatment is endoscopic retrograde cholangiography (ERC) in patients with duct-to-duct (DD) anastomosis and PTC in patients with BE anastomosis (Figure 1). The diagnostic algorithm for BS is shown in Figure 2.

NAS

NAS concerns strictures located in both the extrahepatic and intrahepatic biliary systems of the liver graft[20]. The most severe forms of NAS evolve in the case of early HAT and result in partial or complete biliary necrosis. In the case of late HAT or arterial stenosis, the forms are attenuated due to collateral perfusion. Thus, the severity of NAS correlates with the time of manifestation and is most severe in the first year, whereas late HAT may be even clinically unapparent[12]. A patient with NAS has a cholestatic picture with episodes of cholangitis. More than 50% of the cases present in the first year.

NAS can also develop with an open artery, which represents a distinct entity generally referred to as ischemic-type biliary lesions (ITBL)[20]. NAS can be classified as extrahepatic (type Ⅰ), intrahepatic (type Ⅱ), or a combination of both. NAS can also be sub-classified according to the etiology: NAS secondary to HAT; ITBL secondary to microangiopathic injury (donor factors-preservation injury, prolonged ischemia times, donor after cardiac death); or ITBL secondary to immunogenetic injury (ABO incompatibility, rejection, autoimmune hepatic disease, CMV infection and chemokine polymorphisms)[20].

The incidence of NAS varies between 5% and 25%. Recently, many centers have had an increased incidence due to the more liberal use of extended criteria donors and donors after cardiac death[12]. This is rarely the case in pediatric liver transplantation, where donor selection follows more strict criteria. The rate of NAS after living donor liver transplantation (LDLT) is low, and the cases are usually represented on PSC recurrence.

Risk factors for NAS include the use of UW-solution (with a high-viscosity), Roux-en-Y reconstruction, postoperative CMV infection and PSC as underlying liver disease, cold ischemia time > 10 h, donor age and organ quality (severe macrovascular steatosis)[12].
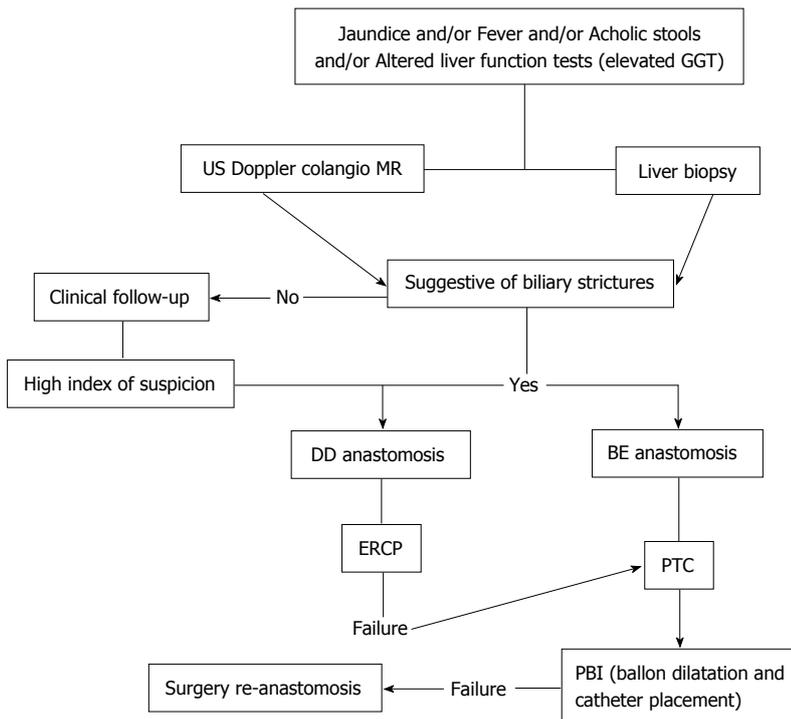
Figure 2 Proposed diagnostic and treatment algorithm for biliary strictures. MR: Magnetic resonance; BE: Bilioenteric; PBI: Percutaneous biliary interventions; PTC: Percutaneous transhepatic cholangiography; DD: Duct-to-duct; US: Ultrasonography; GGT: Gamma-glutamyl transferase; ERCP: Endoscopic retrograde cholangio pancreatography.
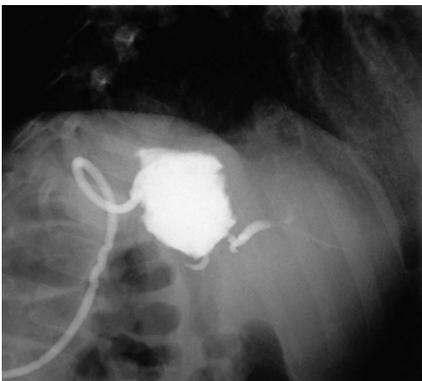
Figure 3 A 3-year-old boy developed a biloma 12-mo after receiving left lateral segment from a live-donor. He had a persistent bile leak and segmental bile duct dilatation on abdominal computed tomography scan imaging. A fistulography was performed by injecting contrast through the pigtail catheter, and an excluded bile duct was evidenced.

HAT is a direct cause of NAS because the blood supply to the biliary tree is almost solely arterial and receives no significant contribution from the portal vein in physiological conditions. However, some re-searchers support the hypothesis that the peribiliary vascular plexus is sustained not only by blood from the hepatic artery but also by blood from the portal vein. Because ITBLs can occur in the absence of HAT, it has been suggested that the portal blood impacts the pathogenesis of ITBL. Patients with partial portal vein thrombosis and intact arterial blood supply have developed ITBL in the segments affected by portal vein thrombosis[20].

MISSING DUCTS

A missing duct or excluded segmental bile duct is a segmental bile duct that is discontinuous with the primary biliary drainage tree of a partial allograft (Figure 3). Its incidence after LDLT can reach 40%[21]. Signs suggestive of a missing duct are persistent bile leak, high direct hyperbilirubinemia and imaging evidence of an isolated dilated bile duct system by US, CT or MR[22]. Additionally, a missing duct should always be suspected when a patient presents signs of cholestasis but has normal stool coloration[4].

Partial grafts always have a cut surface area and occasionally present multiple bile ducts. During the liver resection procedure in live-donor, split or reduced grafts, these secondary bile ducts may be missed and ligated unintentionally. Anatomical variations in biliary drainage exist in which a biliary branch does not communicate with its segmental bile duct and thus drains into another segment[22]. Conzen *et al*[22] reported four cases of excluded bile ducts after pediatric liver transplantation. All of these patients had their diagnosis confirmed by PTC and initially treated by PBI with external catheter placement. Subsequently, a surgical intervention was performed to include this excluded duct in the BE anastomosis. The drainage catheter was used for guidance and remained postoperatively. All but one patient recovered well. The remaining patient developed ductopenic rejection and underwent re-transplantation[22].

Some important measures can help prevent this complication, such as an adequate preoperative eva-

luation of the live donor's biliary tree with a cholangio MR or by means of an intraoperative cholangiography. Additionally, for split and reduced grafts, backtable cholangiography could help identify these secondary ducts[22].

TREATMENT

Treatment strategies for BCs are based on the type and severity of the complication and the biliary reconstruction technique applied (DD or BE anastomosis). Non-operative management is the first-line approach, and success can be achieved in 70%-90% of all BS cases[2,4-6].

BL can be treated conservatively by maintaining the abdominal drain if the patient's condition is stable[4]. Cut surface leaks or small caudate duct leaks usually respond to the conservative approach and close in 5-8 wk[4,7]. Anastomotic leaks, however, require additional intervention. Patients with DD anastomosis are treated with ERC, with reported success rates of 80%-90%[23]. Small leaks can be managed by sphincterotomy alone. ERC management of significant anastomotic BLs is successful approximately 50% of the time, but the remainder of cases requires surgical revision. PTC is used as a second-line therapy, *i.e.*, as a rescue procedure, in patients with DD anastomosis. Early leaks in a clinically unstable patient demand urgent surgical revision. They are usually due to a large defect or even biliary necrosis[11]. Ultimately, most BL will require surgery[4]. Bilomas are usually self-limiting and may be treated by insertion of a percutaneous pigtail catheter. Using all the available resources, the success rates of BL treatment are approximately 85%-100%[12].

Overall 60%-90% of AS can be treated with intervention. In the long term, approximately 10%-20% of such cases require surgical revision. In most centers, surgical intervention is left as last resource for treating AS. Patients with DD anastomosis are approached with ERC with balloon dilatation with or without stent placement[12]. A successful endoscopic treatment can be achieved in 58%-76% of LDLT cases and 80%-90% of deceased donor cases[24]. The complication rate is of 6.6% per procedure, with a cumulative complication rate per patient of 21%[15]. There is less cumulative experience with ERC in children. Dechêne *et al*[25] reported on 17 children with BC submitted to ERC. All but one patient completed the exam, and those patients were treated with sphincterotomy, balloon dilatation or stent placement. Although the most common complication was bleeding (23.5%), only one required surgical revision. In those patients with AS, the success rate was 100%[25].

Unlike most centers, Darius *et al*[26] indicated surgery as the first treatment for AS. The primary patency of surgically treated patients was 80%. The other 20% had recurrences and needed a second surgical intervention. The 30-d mortality was 3.4%; 8.5% had major complications, and most required another surgical intervention[26].

The gold-standard treatment for patients with BE anastomosis is PTC with PBI. This is the mainstay of treatment for liver-transplanted children because the vast majority of these children have a BE anastomosis. With an aggressive interventional radiology team, PTC can be successful 76%-89% of the time[2]. To achieve good results, an early indication of the procedure, based on a high index of clinical suspicion, is paramount. PTC can be performed safely, even in the absence of dilated bile ducts. Most of the procedures performed in our institution are in children who are recipients of partial grafts and lack bile duct dilatation. All such procedures are performed under either US guidance or fluoroscopic control[4]. Percutaneous treatment of AS in pediatric patients is considered safe and effective, and in most cases, surgical revision of the anastomosis is not needed[6]. Contraindications to PTC are relative and include uncorrectable coagulopathy, allergy to iodinated contrast, and large volume ascites. To perform the procedure safely, the patient should have an INR $\leqslant$ 1.5, platelet count $\geqslant$ 50000/dL and a normal partial thromboplastin time[27]. Miraglia *et al*[6], studying pediatric liver transplant recipients with BS, achieved a trans-stricture biliary catheter placement success rate of 92%. During follow-up, 11% of their patients needed surgical revision of the anastomosis. In 75% of the patients, only one course of treatment was required, 20% required two courses and 5% required three courses of percutaneous stenting and bilioplasty. No life-threatening complications were reported[6].

Moreira *et al*[13] studied pediatric liver transplant recipients who underwent PTC due to clinical suspicion of BS. They found that 29.7% had normal findings, 15.6% had simultaneous intrahepatic stenosis and 54.7% had isolated AS. One course of percutaneous treatment section was needed in 65.7% of the patients, two courses were required in 20%, three courses were needed in 11.4% and more than three courses were needed in 2.8%. Thirty-four precent had a recurrence at a median 2.2 years of follow-up. At the end of follow-up, 82.6% were symptom-free. Only two patients presented with hemobilia associated with hemodynamic repercussions and were treated successfully by arterial embolization[13]. Some series reported complication rates of approximately 40%[28]. The most common potential complications from PBI included bleeding, fever, bacteremia and sepsis. Minor complications occurred in approximately 11% of cases, and major complications occurred in less than 2%[27].

For those patients who fail ERC and PTC, a novel magnetic compression anastomosis can be created. Transmural compression with two magnets causes gradual ischemic necrosis, thus creating a new anastomosis between the dilated duct and small intestine or bile duct. There are only few cases in which this technique was performed, and further experience is required before it has broader indications[29,30].

There is still no randomized controlled trial for the

treatment of AS in pediatric liver transplant recipients. PBI is less invasive and has a lower complication rate. The treatment, however, is long, and the patient needs multiple re-interventions for catheter exchange. A direct surgical approach is obviously more invasive and has higher complication rates, though it does have a shorter treatment period[31]. Quality of life cannot currently be evaluated for each treatment approach based on the personal impressions of the attendants. Most centers are more likely to adopt a less invasive strategy than a more invasive strategy[32].

Treatment of NAS is multidisciplinary, and the success rates with interventional treatment are lower than those observed for anastomotic complications. The involvement of the biliary system is diffuse, and severe forms with cast formation do not respond to endoscopic treatment. Usually, ursodeoxycholic acid is used to increase bile flow and lower lithogenicity. Antibiotic therapy and prophylaxis are often necessary. The difficulty in treating this condition is expressed by the 10-year graft failure rates that can occur in 20%-50% of cases[12].

TECHNICAL VARIANT GRAFTS

Technical variant grafts are becoming the most used types of liver grafts in pediatric recipients. However, recipients of technical variant grafts are more likely to develop any type of complication within 30 d than are whole organ recipients. In the study by Diamond *et al*[1], the incidence of BC within 30 d was 18.8% for split livers, 17.5% for LDLT, 16% for reduced liver grafts and 7.5% for whole liver grafts, and the BC were mostly represented by BL. As for the complications observed at 2 years of follow-up, all of the variant grafts were associated with an increased incidence of BC, and all cases presented with an increased incidence of BL. Recipients of reduced and live-donor grafts also had an increased incidence of intrahepatic BS. Recipients of live-donor grafts had a 2-fold increased incidence of AS[1]. However, no other groups have linked an increased incidence of BC with technical variant grafts and claim that the initial high rate of BC decreases after the initial learning experience[9].

LDLT

The incidence of BC after pediatric LDLT ranges from 4% to 45%[10,33]. BC occur more frequently after LDLT than deceased donor transplantation and remain the most common and prolonged treatment problem after LDLT[24]. Reding *et al*[34] reported an incidence of BC of 34% in LDLT *vs* 14% in deceased donors. The incidence of strictures in LDLT is twice that of deceased donors (24% *vs* 12%, respectively)[34].

Donor surgery
Some aspects of the donor surgery are particularly important, and actions toward refining this technique

to lower complication rates in the recipients were taken by groups with large experience in live donation[35]. It is not unusual to have more than one bile duct in the liver graft from either right lobes or left lateral segment grafts (LLS): 40% of LLS grafts require at least 2 biliary anastomosis[9]. It is a common practice to study the donor's biliary anatomy before electing for surgery and/ or during the hepatectomy by means of a transcystic cholangiography. Intraoperative cholangiography can help identify the exact point of bile duct transection. Additionally, preoperative cholangio MR should be performed in the donors, especially in the case of left and right grafts. Cholangio MR accurately described the anatomy in 88.3% of the donors[36]. Another method, by CT cholangiography could accurately define the anatomy in 96% of the donors[37].

An aberrant biliary anatomy and the presence of two or more ducts are significant risk factors for the development of BCs[24]. Generally, donation is precluded if three or more small bile ducts are present. Care should also be taken with the caudate lobe anatomy because BL originating from the caudate lobe are not infrequent and are difficult to manage. A maximum of 5 bile ducts are encountered in the caudate lobe and careful attention should be paid to the performance of continuous suturing and ligation of these radicles[24].

Because ischemia of the bile ducts plays an important role in the development of BC, preserving the blood supply to the bile duct is an important factor to prevent these complications. In LDLT, interruption of the blood supply is thought to be the most important contributing factor for the higher incidence of BC in this population. In a partial liver graft, the blood vessels to the graft bile duct from the common bile duct side are transected. The dissection of the right or left hepatic artery in the donor operation should be restricted to what is absolutely necessary to protect the branches, and the dissection of the hilar duct should be minimal or even avoided. Following the principle of preserving the bile duct blood supply in the donor, Soin *et al*[35] applied a complete Glissonian approach in the donor's hepatectomy and reduced the incidence and severity of BC in the recipients from 15.8% to 5.3%.

RECIPIENT OPERATION: UNSOLVED TECHNICAL DILEMMAS AND FUTURE TRENDS

In the beginning of the LDLT experience, the standard biliary reconstruction procedure was the Roux-en-Y hepaticojejunostomy. It is also the preferred method for children because their bile ducts are too small or the BE anastomosis is mandated because of underlying liver disease. In adult LDLT, the routine use of a DD reconstruction is now applied because of its theoretical advantages: it preserves the sphincter mechanism, decreases the operative time and allows access through ERC[24]. However, it cannot always be performed, as

Table 2 Different biliary reconstruction techniques in pediatric liver transplantation and biliary complications incidence

Ref.	*N*	Type of graft	BE/DD	Suture technique	Stent	BC	BS	BL
Okajima *et al*[38]	6	LDLT	0/6	Interrupted	Yes	16.6%	16.6%	0
Sakamoto *et al*[12]	19	LDLT	0/19	Continuous and interrupted	Yes, but not routine	47.4%	36.8%	10.5%
Shirouzu *et al*[3]	30	LDLT	20/10	Interrupted	Yes	6.6%	3.3%	3.3%
Liu *et al*[10]	7	LDLT	3/4	Interrupted	No	14.2%	0	14.2%
Anderson *et al*[5]	66	Whole, split and reduced	51/15	Continuous and interrupted	No	26%	23%	3%
Tanaka *et al*[37]	60	LDLT	46/14	Continuous and interrupted/only interrupted	Yes/No	20%	11.7%	5%
Haberal *et al*[39]	31	LDLT	0/31	-	No	15.6%	9.3%	6.2%
Ando *et al*[9]	49	LDLT	47/2	Interrupted, wide interval	Yes	4%	2%	2%
Chok *et al*[40]	78	LDLT	74/4	Continuous posterior/interrupted anterior	No		16.7%	
Feier *et al*[4]	489	LDLT	-	Continuous and interrupted	No	14.5%	9.2%	6.7%
Darius *et al*[30]	429	Whole, split, reduced and LDLT	395/24	Interrupted	No	23%	13.2%	3.0%

BE: Bilioenteric anastomosis; DD: Duct-to-duct anastomosis; BC: Biliary complication; BS: Biliary stricture; BL: Bile leak; LDLT: Living donor liver transplant.

in cases of PSC, biliary atresia, duct size mismatch, tension and twisting of the bile duct as in partial liver grafts.

In recent years, more pediatric groups are adopting the DD reconstruction after pediatric liver transplantation whenever it is technically possible, and some advocate that it provides better outcomes than the BE anastomosis. Tanaka *et al*[38] compared the results of the two techniques in 60 pediatric LDLT recipients. The overall BC incidence was 20%. Patients in the BE group had more BL (6.5% *vs* 0%), but patients in the DD group had more strictures (21.4% *vs* 8.7%). The researchers could not confirm the advantage of the DD anastomosis and noted that when a stricture developed, it was more difficult to treat if the patient had a DD anastomosis; however, ERCP was not applied to treat these patients[38]. Liu *et al*[11] also recognized problems when performing a DD anastomosis: three patients had to be converted to a BE anastomosis because of kinking and tension. In a smaller cohort, Shirouzu *et al*[3] showed no difference in the incidence of complications between the techniques. Sakamoto *et al*[33] reported on 19 pediatric recipients with a DD anastomosis and observed an incidence of BC of 47.4%. They argued that when the biliary stent was routinely placed, the incidence of complications dropped; thus, they recommended the use of stents when performing a DD anastomosis[33].

To date, there is no high-impact study that has proved the superiority of a DD anastomosis in children. Because endoscopic biliary intervention is not widely performed in younger children because of its technical difficulty, the choice between a BE or DD anastomosis should not be driven by the performance of ERC in children. A common problem encountered with the DD in partial liver grafts is the kinking of the anastomosis due to tension during the liver transplant or during follow-up because of the regeneration process. The BE anastomosis may be less susceptible to kinking because the intestinal loop has more mobility[38]. Generally, in patients with a preserved bile duct, such as those with metabolic diseases, liver tumors, cryptogenic cirrhosis an

attempt could be made to perform a DD anastomosis. Bile duct size mismatch, tension and kinking of the anastomosis may preclude the performance of a DD, especially in partial liver grafts, because it increases the risk of a bile duct stricture.

Another regularly raised issue is the use of anastomotic stents. The rationale for a stent is the maintenance of the biliary flow despite swelling of the anastomosis as well as easy access for control cholangiography in case of a suspected leak or stricture. However, the stent itself is a foreign body and can induce inflammation and subsequent stricture[24]. Several groups with experience in pediatric liver transplantation have a great variety of techniques for reconstructing the biliary duct[3-5,10,11,26,38-41] (Table 2). It would take a large prospective controlled study to define the best technique for biliary reconstruction.

Microsurgical techniques are emerging as an alternative to lower the incidence of BC in LDLT. Lin *et al*[42] started applying microsurgical techniques to biliary reconstruction in LDLT. Their first report showed comparable results between the conventional and microsurgical groups, with overall complication rates of 18.8% and 15.3%, respectively. However, after stratifying the cases and excluding the learning curve, the results with the microsurgical technique improved with an overall incidence of BC of 5.4%[42]. Their long-term results were published later, and the incidence of BC was 9.6%. Their sample was constituted mainly of adult patients, right lobe grafts and single duct openings[43]. Further experience with this type of reconstruction in children with a BE anastomosis could help define the role of microsurgery in this subgroup of patients.

CONCLUSION

Despite difficult diagnosis and prolonged treatment, BS have a high rate of resolution with non-operative management. BL will ultimately require surgical treatment unless it is caused by a cut surface leak. No

significant difference was observed regarding patient or graft survival in the different series[2,4,26]. However, the presence of BC, particularly BL increases the length of stay and hospital costs[2,44]. Technical refinement, especially in technical variant grafts, might be the key to lowering the incidence of BC in pediatric liver transplant recipients.

REFERENCES

1   **Diamond IR**, Fecteau A, Millis JM, Losanoff JE, Ng V, Anand R, Song C; SPLIT Research Group. Impact of graft type on outcome in pediatric liver transplantation: a report From Studies of Pediatric Liver Transplantation (SPLIT). *Ann Surg* 2007; **246**: 301-310 [PMID: 17667510 DOI: 10.1097/SLA.0b013e3180caa415]

2   **Kling K**, Lau H, Colombani P. Biliary complications of living related pediatric liver transplant patients. *Pediatr Transplant* 2004; **8**: 178-184 [PMID: 15049799 DOI: 10.1046/j.1399-3046.2003.00127.x]

3   **Shirouzu Y**, Okajima H, Ogata S, Ohya Y, Tsukamoto Y, Yamamoto H, Takeichi T, Kwang-Jong L, Asonuma K, Inomata Y. Biliary reconstruction for infantile living donor liver transplantation: Roux-en-Y hepaticojejunostomy or duct-to-duct choledochocholedochostomy? *Liver Transpl* 2008; **14**: 1761-1765 [PMID: 19025922 DOI: 10.1002/lt.21599]

4   **Feier FH**, Chapchap P, Pugliese R, da Fonseca EA, Carnevale FC, Moreira AM, Zurstrassen C, Santos AC, Miura IK, Baggio V, Porta A, Guimarães T, Cândido H, Benavides M, Godoy A, Leite KM, Porta G, Kondo M, Seda-Neto J. Diagnosis and management of biliary complications in pediatric living donor liver transplant recipients. *Liver Transpl* 2014; **20**: 882-892 [PMID: 24760734 DOI: 10.1002/lt.23896]

5   **Anderson CD**, Turmelle YP, Darcy M, Shepherd RW, Weymann A, Nadler M, Guelker S, Chapman WC, Lowell JA. Biliary strictures in pediatric liver transplant recipients - early diagnosis and treatment results in excellent graft outcomes. *Pediatr Transplant* 2010; **14**: 358-363 [PMID: 20003138 DOI: 10.1111/j.1399-3046.2009.01246.x]

6   **Miraglia R**, Maruzzelli L, Caruso S, Riva S, Spada M, Luca A, Gridelli B. Percutaneous management of biliary strictures after pediatric liver transplantation. *Cardiovasc Intervent Radiol* 2008; **31**: 993-998 [PMID: 18574628 DOI: 10.1007/s00270-008-9378-5]

7   **Ostroff JW**. Management of biliary complications in the liver transplant patient. *Gastroenterol Hepatol* (N Y) 2010; **6**: 264-272 [PMID: 20567581]

8   **Gupta S**, Castel H, Rao RV, Picard M, Lilly L, Faughnan ME, Pomier-Layrargues G. Improved survival after liver transplantation in patients with hepatopulmonary syndrome. *Am J Transplant* 2010; **10**: 354-363 [PMID: 19775311 DOI: 10.1111/j.1600-6143.2009.02822.x]

9   **Salvalaggio PR**, Whitington PF, Alonso EM, Superina RA. Presence of multiple bile ducts in the liver graft increases the incidence of biliary complications in pediatric liver transplantation. *Liver Transpl* 2005; **11**: 161-166 [PMID: 15666393 DOI: 10.1002/lt.20288]

10 **Ando H**, Kaneko K, Ono Y, Tainaka T, Kawai Y. Biliary reconstruction with wide-interval interrupted suture to prevent biliary complications in pediatric living-donor liver transplantation. *J Hepatobiliary Pancreat Sci* 2011; **18**: 26-31 [PMID: 20602241 DOI: 10.1007/s00534-010-0301-5]

11 **Liu C**, Loong CC, Hsia CY, Peng CK, Tsai HL, Tsou MY, Wei C. Duct-to-duct biliary reconstruction in selected cases in pediatric living-donor left-lobe liver transplantation. *Pediatr Transplant* 2009; **13**: 693-696 [PMID: 19067924 DOI: 10.1111/j.1399-3046.2008.01040.x]

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

13 **Moreira AM**, Carnevale FC, Tannuri U, Suzuki L, Gibelli N, Maksoud JG, Cerri GG. Long-term results of percutaneous bilioenteric anastomotic stricture treatment in liver-transplanted children. *Cardiovasc Intervent Radiol* 2010; **33**: 90-96 [PMID: 19543942 DOI: 10.1007/s00270-009-9619-2]

14 **Teplisky D**, Urueña Tincani E, Halac E, Garriga M, Cervio G, Imventarza O, Sierre S. Ultrasonography, laboratory, and cholangiography correlation of biliary complications in pediatric liver transplantation. *Pediatr Transplant* 2015; **19**: 170-174 [PMID: 25529070 DOI: 10.1111/petr.12421]

15 **Holt AP**, Thorburn D, Mirza D, Gunson B, Wong T, Haydon G. A prospective study of standardized nonsurgical therapy in the management of biliary anastomotic strictures complicating liver transplantation. *Transplantation* 2007; **84**: 857-863 [PMID: 17984838 DOI: 10.1097/01.tp.0000282805.33658.ce]

16 **Potthoff A**, Hahn A, Kubicka S, Schneider A, Wedemeyer J, Klempnauer J, Manns M, Gebel M, Boozari B. Diagnostic value of ultrasound in detection of biliary tract complications after liver transplantation. *Hepat Mon* 2013; **13**: e6003 [PMID: 23483295 DOI: 10.5812/hepatmon.6003]

17 **Katz LH**, Benjaminov O, Belinki A, Geler A, Braun M, Knizhnik M, Aizner S, Shaharabani E, Sulkes J, Shabtai E, Pappo O, Atar E, Tur-Kaspa R, Mor E, Ben-Ari Z. Magnetic resonance cholangiopancreatography for the accurate diagnosis of biliary complications after liver transplantation: comparison with endoscopic retrograde cholangiography and percutaneous transhepatic cholangiography - long-term follow-up. *Clin Transplant* 2010; **24**: E163-E169 [PMID: 21039885 DOI: 10.1111/j.1399-0012.2010.01300.x]

18 **Kinner S**, Dechêne A, Paul A, Umutlu L, Ladd SC, de Dechêne EM, Zöpf T, Gerken G, Lauenstein TC. Detection of biliary stenoses in patients after liver transplantation: is there a different diagnostic accuracy of MRCP depending on the type of biliary anastomosis? *Eur J Radiol* 2011; **80**: e20-e28 [PMID: 20580506 DOI: 10.1016/j.ejrad.2010.06.003]

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

20 **Cursio R**, Gugenheim J. Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation. *J Transplant* 2012; **2012**: 164329 [PMID: 22530107 DOI: 10.1155/2012/164329]

21 **Huang TL**, Cheng YF, Chen CL, Chen TY, Lee TY. Variants of the bile ducts: clinical application in the potential donor of living-related hepatic transplantation. *Transplant Proc* 1996; **28**: 1669-1670 [PMID: 8658831]

22 **Conzen KD**, Lowell JA, Chapman WC, Darcy M, Duncan JR, Nadler M, Turmelle YP, Shepherd RW, Anderson CD. Management of excluded bile ducts in paediatric orthotopic liver transplant recipients of technical variant allografts. *HPB* (Oxford) 2011; **13**: 893-898 [PMID: 22081926 DOI: 10.1111/j.1477-2574.2011.00394.x]

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

24 **Wang SF**, Huang ZY, Chen XP. Biliary complications after living donor liver transplantation. *Liver Transpl* 2011; **17**: 1127-1136 [PMID: 21761548 DOI: 10.1002/lt.22381]

25 **Dechêne A**, Kodde C, Kathemann S, Treckmann J, Lainka E, Paul A, Gerken G, Feldstein AE, Hoyer PF, Canbay A. Endoscopic treatment of pediatric post-transplant biliary complications is safe and effective. *Dig Endosc* 2015; **27**: 505-511 [PMID: 25545826 DOI: 10.1111/den.12420]

26 **Darius T**, Rivera J, Fusaro F, Lai Q, de Magnée C, Bourdeaux

C, Janssen M, Clapuyt P, Reding R. Risk factors and surgical management of anastomotic biliary complications after pediatric liver transplantation. *Liver Transpl* 2014; **20**: 893-903 [PMID: 24809592 DOI: 10.1002/lt.23910]

27 **Racadio JM**, Kukreja K. Pediatric biliary interventions. *Tech Vasc Interv Radiol* 2010; **13**: 244-249 [PMID: 21055679 DOI: 10.1053/j.tvir.2010.04.007]

28 **Migliazza L**, López Santamaría M, Murcia J, Gamez M, Clavijo J, Camarena C, Hierro L, Frauca E, de la Vega A, Diaz M, Jara P, Tovar JA. Long-term survival expectancy after liver transplantation in children. *J Pediatr Surg* 2000; **35**: 5-7; discussion 7-8 [PMID: 10646764 DOI: 10.1016/S0022-3468(00)80003-2]

29 **Marubashi S**, Nagano H, Yamanouchi E, Kobayashi S, Eguchi H, Takeda Y, Tanemura M, Maeda N, Tomoda K, Hikita H, Tsutsui S, Doki Y, Mori M. Salvage cystic duct anastomosis using a magnetic compression technique for incomplete bile duct reconstruction in living donor liver transplantation. *Liver Transpl* 2010; **16**: 33-37 [PMID: 20035518 DOI: 10.1002/lt.21934]

30 **Muraoka N**, Uematsu H, Yamanouchi E, Kinoshita K, Takeda T, Ihara N, Matsunami H, Itoh H. Yamanouchi magnetic compression anastomosis for bilioenteric anastomotic stricture after living-donor liver transplantation. *J Vasc Interv Radiol* 2005; **16**: 1263-1267 [PMID: 16151070 DOI: 10.1097/01.RVI.0000173280.56442.9E]

31 **Darius T**, Reding R. Therapeutic strategy for anastomotic biliary strictures after pediatric liver transplantation: two radically different approaches. *Liver Transpl* 2014; **20**: 876-878 [PMID: 24943482 DOI: 10.1002/lt.23933]

32 **Seda Neto J**, Chapchap P. When is surgery required for the treatment of biliary complications after pediatric liver transplantation? *Liver Transpl* 2014; **20**: 879-881 [PMID: 24943332 DOI: 10.1002/lt.23932]

33 **Sakamoto S**, Egawa H, Ogawa K, Ogura Y, Oike F, Ueda M, Yazumi S, Shibata T, Takada Y, Uemoto S. The technical pitfalls of duct-to-duct biliary reconstruction in pediatric living-donor left-lobe liver transplantation: the impact of stent placement. *Pediatr Transplant* 2008; **12**: 661-665 [PMID: 18093087 DOI: 10.1111/j.1399-3046.2007.00870.x]

34 **Reding R**, de Goyet Jde V, Delbeke I, Sokal E, Jamart J, Janssen M, Otte JB. Pediatric liver transplantation with cadaveric or living related donors: comparative results in 90 elective recipients of primary grafts. *J Pediatr* 1999; **134**: 280-286 [PMID: 10064662 DOI: 10.1016/S0022-3476(99)70450-6]

35 **Soin AS**, Kumaran V, Rastogi AN, Mohanka R, Mehta N, Saigal S, Saraf N, Mohan N, Nundy S. Evolution of a reliable biliary reconstructive technique in 400 consecutive living donor liver transplants. *J Am Coll Surg* 2010; **211**: 24-32 [PMID: 20610245 DOI: 10.1016/j.jamcollsurg.2010.02.048]

36 **Song GW**, Lee SG, Hwang S, Sung GB, Park KM, Kim KH, Ahn CS, Moon DB, Ha TY, Kim BS, Moon KM, Jung DH. Preoperative evaluation of biliary anatomy of donor in living donor liver transplantation by conventional nonenhanced magnetic resonance cholangiography. *Transpl Int* 2007; **20**: 167-173 [PMID: 17239025 DOI: 10.1111/j.1432-2277.2006.00419.x]

37 **Wang ZJ**, Yeh BM, Roberts JP, Breiman RS, Qayyum A, Coakley FV. Living donor candidates for right hepatic lobe transplantation: evaluation at CT cholangiography--initial experience. *Radiology* 2005; **235**: 899-904 [PMID: 15833987 DOI: 10.1148/radiol.2353040424]

38 **Tanaka H**, Fukuda A, Shigeta T, Kuroda T, Kimura T, Sakamoto S, Kasahara M. Biliary reconstruction in pediatric live donor liver transplantation: duct-to-duct or Roux-en-Y hepaticojejunostomy. *J Pediatr Surg* 2010; **45**: 1668-1675 [PMID: 20713218 DOI: 10.1016/j.jpedsurg.2010.03.010]

39 **Okajima H**, Inomata Y, Asonuma K, Ueno M, Ishiko T, Takeichi T, Kodera A, Yoshimoto K, Ohya Y. Duct-to-duct biliary reconstruction in pediatric living donor liver transplantation. *Pediatr Transplant* 2005; **9**: 531-533 [PMID: 16048608 DOI: 10.1111/j.1399-3046.2005.00336.x]

40 **Haberal M**, Karakayali H, Atiq A, Sevmis S, Moray G, Ozcay F, Boyvat F. Duct-to-duct biliary reconstruction without a stent in pediatric living-donor liver transplantation. *Transplant Proc* 2011; **43**: 595-597 [PMID: 21440772 DOI: 10.1016/j.transproceed.2011.01.009]

41 **Chok KS**, Chan SC, Chan KL, Sharr WW, Tam PK, Fan ST, Lo CM. Bile duct anastomotic stricture after pediatric living donor liver transplantation. *J Pediatr Surg* 2012; **47**: 1399-1403 [PMID: 22813803 DOI: 10.1016/j.jpedsurg.2011.12.014]

42 **Lin TS**, Concejero AM, Chen CL, Chiang YC, Wang CC, Wang SH, Liu YW, Yang CH, Yong CC, Jawan B, Cheng YF. Routine microsurgical biliary reconstruction decreases early anastomotic complications in living donor liver transplantation. *Liver Transpl* 2009; **15**: 1766-1775 [PMID: 19938121 DOI: 10.1002/lt.21947]

43 **Lin TS**, Chen CL, Concejero AM, Yap AQ, Lin YH, Liu CY, Chiang YC, Wang CC, Wang SH, Lin CC, Yong CC, Cheng YF. Early and long-term results of routine microsurgical biliary reconstruction in living donor liver transplantation. *Liver Transpl* 2013; **19**: 207-214 [PMID: 23197399 DOI: 10.1002/lt.23582]

44 **Englesbe MJ**, Dimick J, Mathur A, Ads Y, Welling TH, Pelletier SJ, Heidt DG, Magee JC, Sung RS, Punch JD, Hanto DW, Campbell DA. Who pays for biliary complications following liver transplant? A business case for quality improvement. *Am J Transplant* 2006; **6**: 2978-2982 [PMID: 17294525 DOI: 10.1111/j.1600-6143.2006.01575.x]

MINIREVIEWS

Current status of preoperative drainage for distal biliary obstruction

Harutoshi Sugiyama, Toshio Tsuyuguchi, Yuji Sakai, Rintaro Mikata, Shin Yasui, Yuto Watanabe, Dai Sakamoto, Masato Nakamura, Reina Sasaki, Jun-ichi Senoo, Yuko Kusakabe, Masahiro Hayashi, Osamu Yokosuka

Harutoshi Sugiyama, Toshio Tsuyuguchi, Yuji Sakai, Rintaro Mikata, Shin Yasui, Yuto Watanabe, Dai Sakamoto, Masato Nakamura, Reina Sasaki, Jun-ichi Senoo, Yuko Kusakabe, Masahiro Hayashi, Osamu Yokosuka, Department of Gastroenterology and Nephrology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

Author contributions: Sugiyama H substantially contributed to the study design and writing of the manuscript; Tsuyuguchi T contributed to the critical revision of the manuscript and the approval of the article to be published; Sakai Y contributed to the revision of the manuscript and to the revision of the review; Mikata R, Yasui S, Watanabe Y and Sakamoto D contributed to the revision of the manuscript; Nakamura M, Sasaki R, Senoo J, Kusakabe Y and Hayashi M contributed to the revision of the manuscript and to the revision of the English; Yokosuka O contributed to the final approval of the version of the article to be published.

Conflict-of-interest statement: The authors report no disclosures relevant to this publication.

Correspondence to: Harutoshi Sugiyama, MD, PhD, Assistant Professor, Department of Gastroenterology and Nephrology, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuou-ku, Chiba 260-8670, Japan. sugi1.1220@gmail.com
Telephone: +81-43-2262083
Fax: +81-43-2262088

Abstract

Preoperative biliary drainage (PBD) was developed to improve obstructive jaundice, which affects a number of organs and physiological mechanisms in patients waiting for surgery. However, its role in patients who will undergo pancreaticoduodenectomy for biliary obstruction remains controversial. This article aims to review the current status of the use of preoperative drainage for distal biliary obstruction. Relevant articles published from 1980 to 2015 were identified by searching MEDLINE and PubMed using the keywords "PBD", "pancreaticoduodenectomy", and "obstructive jaundice". Additional papers were identified by a manual search of the references from key articles. Current studies have demonstrated that PBD should not be routinely performed because of the postoperative complications. PBD should only be considered in carefully selected patients, particularly in cases where surgery had to be delayed. PBD may be needed in patients with severe jaundice, concomitant cholangitis, or severe malnutrition. The optimal method of biliary drainage has yet to be confirmed. PBD should be performed by endoscopic routes rather than by percutaneous routes to avoid metastatic tumor seeding. Endoscopic stenting or nasobiliary drainage can be selected. Although more expensive, the use of metallic stents remains a viable option to achieve effective drainage without cholangitis and reintervention.

Key words: Preoperative drainage; Biliary drainage; Distal biliary obstruction; Pancreaticoduodenectomy; Obstructive jaundice

Core tip: Because of the postoperative complications, studies have demonstrated that preoperative biliary drainage (PBD) should not be routinely performed in patients who will undergo pancreaticoduodenectomy. PBD may be selectively applied in patients with severe jaundice, cholangitis, or severe malnutrition and in those patients with a relatively long wait before surgery. PBD should be performed through endoscopic routes rather than percutaneous routes to avoid metastatic tumor seeding. Endoscopic stenting or nasobiliary drainage can be selected. Although more expensive, the use of metallic stents remains a viable option to avoid reinterventions.

INTRODUCTION

Surgical resection is generally considered to be the only curative treatment for patients with periampullary cancer or cancer of the pancreatic head. Obstructive jaundice due to distal biliary obstruction is the most common symptom in such patients. Preoperative biliary drainage (PBD) was introduced in the 1970s to relieve the obstruction and to reverse physiological dysfunction resulting from obstructive jaundice. PBD was previously considered to improve surgical outcomes in patients with malignant distal biliary obstruction who were undergoing curative resection, with many physicians electing to perform PBD in patients who were waiting for surgery[1]. A number of PBD methods exist, including endoscopic plastic stenting, nasobiliary drainage, metallic stenting, and percutaneous drainage. However, the overall benefit of PBD is currently controversial.

OVERVIEW

In 2002, a systematic review summarized all prospective and retrospective studies published between 1966 and 2001 to evaluate the efficacy of drainage compared with that of direct surgery in patients with malignant obstructive jaundice[2]. Five randomized controlled studies and 18 cohort studies were analyzed. A meta-analysis of both level I and level II studies found no difference in mortality between patients who underwent PBD and those who underwent surgery without PBD. However, the overall complication rates were significantly and adversely affected by PBD compared with surgery without PBD; for level I studies, the complication rates of the two approaches were 57% and 42%, respectively, indicating a relative reduction of 15% and an absolute risk reduction of 27% in cases where surgery was performed without PBD. Moreover, the overall hospital stay was prolonged following PBD. That meta-analysis concluded that the benefit of PBD did not outweigh the disadvantages of the drainage procedure and complication rates and that PBD should therefore not be routinely performed. Particularly following the publication of this meta-analysis, the routine performance of PBD was apparently no longer recommended. A multicenter, randomized trial was reported in 2010. van der Gaag *et al*[3] compared PBD prior to surgery with surgery alone for patients with cancer of the pancreatic head. They concluded that routine PBD in patients undergoing surgery for cancer of the pancreatic head increased the rate of complications. This report corroborated the results of the previous meta-analysis.

However, the above-mentioned studies have some limitations. The meta-analysis published in 2002 is limited by the fact that not all randomized trials are equal in terms of size or quality. The five randomized trials reviewed were relatively poorly designed, with broad eligibility criteria including both distal and proximal cancers, small sample sizes, different interventions (internal and external drainage) and a number of differing surgical resection procedures. These trials reflect the 1970s approach to obstructive jaundice and surgery, as demonstrated by the higher rates of the use of external percutaneous approaches (59%), lower resection rates (16%), and significantly higher rates of perioperative death (12%).

There are also apparent limitations to the report by van der Gaag *et al*[3]. In that study, the initial rates of endoscopic retrograde cholangiopancreatography (ERCP) failure were 25%, and there were also ERCP-related complications, including pancreatitis, perforation, cholangitis, and bleeding, in 46% of the patients included in the study. Both results appear excessive to us because the majority of studies report rates of approximately 5%-10%[4,5]. Moreover, these authors only used plastic stents, which have been associated with early stent occlusion following cholangitis in up to 26% of patients.

Three other meta-analyses have concluded that PBD does not reduce post-operative mortality and complications in cases of malignant obstructive jaundice and that PBD in patients undergoing surgery for obstructive jaundice is associated with increased serious morbidity; however, these studies included proximal obstruction in addition to distal obstruction[6-8]. Based on another meta-analysis of studies that evaluated the use of PBD in patients who were waiting for pancreaticoduodenectomy, the use of PBD increased postoperative wound infection rates, with no overwhelming evidence that PBD either promoted or protected against other complications. However, a limitation of this report was the lack of comparison between percutaneous transhepatic biliary

drainage (PTBD) and endoscopic drainage[9]. Although no study has yet equally randomized comparable patients according to the level of obstruction and PBD method used, and the results of the previous studies remain controversial, all reported meta-analyses have concluded that PBD should not be performed routinely where possible.

PATIENTS FREQUENTLY UNDERGO PBD BEFORE SURGICAL CONSULTATION

A significant issue with PBD has been the use of the technique before surgical referral or consultation. The first and largest population-based study of patients with pancreatic cancer undergoing pancreaticoduodenectomy at a single institution found that 77% of 2573 patients who were referred to a surgeon already had a stent in place[10]. This result was consistent with previous studies reporting prior stent placement rates of 42%-79%[11-14].

The report concluded that the use of preoperative biliary stenting doubled between 1992 and 2007 despite evidence suggesting that stenting was associated with increased perioperative infectious complications. The performance of PBD prior to surgical consultation can be associated with significant delays in the time to operate in many cases. Therefore, patients waiting for pancreaticoduodenectomy should ideally be carefully treated following discussion between surgeons and endoscopists regarding the necessity of PBD[10].

There are three remaining questions: (1) How do we select patients who are suitable for PBD? (2) What is the appropriate PBD method with minimum complications? and (3) How do we reduce the complications associated with PBD?

HOW DO WE SELECT PATIENTS WITH DISTAL BILIARY OBSTRUCTION WORLD HEALTH ORGANIZATION ARE SUITABLE FOR PBD?

Preoperative drainage should be performed after consideration of the following factors[15,16]: (1) The period of time from diagnosis to anticipated surgery; (2) The presence of an urgent indication for biliary drainage; that is, acute cholangitis, severe pruritus, or severe obstruction with very high bilirubin levels; (3) The functional status of the patient. Many patients are in poor status in terms of nutrition due to obstructive jaundice, which is expected to improve with PBD; and (4) The plan for neoadjuvant chemotherapy or chemoradiation for locally advanced or borderline resectable cancer, where PBD may prevent hepatotoxicity from chemotherapeutic agent.

In 1999, Povoski *et al*[17] reported that PBD was the only factor associated with postoperative infection and postoperative death. Bacterobilia was thought to develop in some patients with biliary stents due to postoperative ascending colonization[18]. Although some reports discourage the use of PBD in cases of distal biliary obstruction[19-24], a recent Cochrane Review found that PBD in patients with resectable pancreatic cancer and periampullary cancer undergoing surgery was associated with a similar mortality rate, but an increased incidence of serious morbidity, compared with patients who did not undergo PBD[25]. Recent studies have reported the effect of selective biliary drainage on perioperative morbidity and mortality in patients undergoing pancreaticoduodenectomy[26,27]. Jagannath *et al*[26] reported that a positive bile culture in patients with drainage was associated with stent complications and duration of stenting and that uncomplicated stenting was not associated with increased rates of serious morbidity or mortality. Coates *et al*[27] also concluded that the morbidity and mortality associated with PBD may not be as significant as previously reported due to recent refinements in endoscopic techniques and improvements in perioperative management.

WHAT IS THE OPTIMAL PBD METHOD WITH MINIMAL COMPLICATIONS?

One of the largest prospective randomized trials, performed in the United States by Pitt *et al*[28], concluded that PTBD does not reduce operative risk, increases hospital cost and is therefore not recommended. In contrast, a recent study reported that PTBD was superior to endoscopic drainage from the perspective of cost-effectiveness; however, the lower cost was related to those patients who were initially subjected to endoscopic drainage and later changed to PTBD[29]. It is also important to determine which method is safer in terms of the long-term survival of patients with resectable distal cancer. Two recent retrospective studies revealed that patients with resectable pancreatic cancer who underwent PTBD had significantly worse survival than patients who underwent endoscopic biliary drainage (EBD)[30,31]. Strom *et al*[30] reported that patients with PTBD had an even worse 5-year survival of just 3%, whereas patients who underwent EBD and patients without PBD had 5-year survival rates of 24% and 32%, respectively. The result was almost identical to the results reported by Murakami *et al*[31]. The major underlying cause of this finding is thought to be metastatic tumor seeding along the PTBD sinus tract.

The main advantage of endoscopic drainage over percutaneous intervention is the avoidance of skin and liver puncture in patients with underlying coagulopathy and the avoidance of tumor seeding along the catheter track. ERCP with biliary drainage has become the first line technique for the treatment of distal biliary obstruction. In addition, ERCP is considered a diagnostic tool in many countries due to the clinical importance of biopsy material or cytology. However, only a few studies have evaluated the safety and efficacy of each method of endoscopic drainage for malignant distal

biliary obstruction. To the best of our knowledge, our study was the first to compare endoscopic biliary stenting (EBS) with endoscopic nasobiliary drainage (ENBD) for PBD in patients with malignant distal biliary obstruction[32]. No significant differences in the overall rate of catheter-related complications, the rate of tube dysfunction, or the median interval from PBD to the time of tube dysfunction were observed between the two groups. Adequate endoscopic PBD was achieved in all patients on the first attempt, and all patients underwent surgery following a successful PBD. Symptoms such as cholangitis and obstructive jaundice resolved within 7 d after the drainage was placed in all patients. Two major complications occurred: one case of cholangitis and another of perforation due to endoscopic sphincterotomy were observed in patients in the EBS group, both of whom recovered following conservative treatment. Another retrospective study demonstrated that EBS increased the rates of wound infection because of a high incidence of cholangitis prior to operative intervention and should, therefore, be avoided[33]. In that study, ENBD had no effect on complication rates. However, this finding required further analysis due to the small number of patients included in this study.

Several studies have reported the utility of self-expanding metallic stents (SEMSs)[15,34,35]. Singal *et al*[34] demonstrated that SEMSs provide excellent patency, with cholangitis occurring in < 5% cases after 4 wk; does not affect surgical technique; and results in minimal postoperative complications in patients waiting to undergo pancreaticoduodenectomy. In a study of 29 patients with pancreatic cancer, Decker *et al*[35] reported no preoperative intervention in the group that received SEMS placement, and up to 40% of the group that underwent plastic stenting required reintervention. The other two studies comparing plastic and metallic stents for internal drainage found no significant difference in either the overall or serious complication rates between SEMSs and plastic stents[36,37]; however, Haapamäki *et al*[37] concluded that the significantly higher price of SEMSs restricts their use to selected cases. A number of recent studies have recommended the use of SEMSs in patients who are candidates for neoadjuvant chemotherapy or chemoradiation with obstructive jaundice and resectable or borderline resectable pancreatic cancer[15,38,39]; however, the small number of prospective studies available means this conclusion remains unreliable.

Another type of stent is completely contained within the bile duct without one end extending to the duodenum. This stent is called an inside stent and is expected to prevent the reflux of intestinal contents into the bile tree. Inside stents have been mainly used for proximal bile duct strictures. Fewer early complications, such as ascending cholangitis, and ease of performing reinterventions were reported[40,41]. Although few reports have evaluated using inside stents for distal biliary obstruction, the latest report demonstrated their efficacy for PBD in biliary tract cancer[42]. Kobayashi *et al*[42]

retrospectively compared the postoperative complications in two groups and reported that morbidity rates were 34.3% (11/32) in the conventional stent group and 24.0% (6/25) in the inside stent group. The limitation of the report was that they included not only patients with distal obstruction but also those with proximal obstruction; additionally, they did not include patients with pancreatic cancer[42].

Rerknimitr *et al*[43] observed that the different types of drainage were used in previous analyses; therefore, the patient groups who underwent internal or external drainages were not homogeneous.

Further randomized studies are needed to determine the optimal method of PBD for distal biliary obstruction.

HOW TO REDUCE POSTOPERATIVE INFECTION?

Despite many recent studies that have concluded that PBD is not associated with increased postoperative infection[44-48], the possibility of preoperative cholangitis caused by tube dysfunction after preoperative drainage remains. Sudo *et al*[49] revealed that the susceptibility of biliary microorganisms to antibiotics was the only independent risk factor for postoperative infections in the largest detailed bacteriologic analysis of intraoperative bile cultures corresponding to PBD procedures using data collected from 254 patients who were undergoing pancreaticoduodenectomy. That study found that perioperative antibiotics covering bile contamination may prevent infectious complications following pancreaticoduodenectomy in patients with and without PBD.

In addition to preoperative cholangitis, drainage duration may also impact patient outcomes through the development of preoperative complications. In general, a minimum of 4-6 wk of PBD is advised. An overly long drainage duration may increase infectious morbidity. Son *et al*[50] reported that a PBD duration of < 2 wk, which was associated with lower rates of preoperative drainage-related complications, was more appropriate in severely jaundiced patients with periampullary cancer. However, the optimal duration in that study may not be appropriate because the study did not strictly distinguish between the different PBD methods. The optimal drainage time will continue to be a controversial issue because neoadjuvant chemotherapy and chemoradiation are currently considered for locally advanced or borderline resectable cancer.

CONCLUSION

The majority of authors believe that PBD should not be routinely performed in patients with malignant distal obstruction due to a possible effect on preoperative complication rates. However, preoperative drainage may be selectively applied in patients with severe jaundice, concomitant cholangitis, or severe malnutrition and in patients who must wait for a relatively long time

before surgery. To avoid complications, PBD should be completed with endoscopic stenting or nasobiliary drainage. Although the cost is significantly higher, metallic stenting can be utilized in patients waiting for surgery for more than 4 wk. Further randomized studies are required to determine the optimal PBD method for distal biliary obstruction.

REFERENCES

1   **Hatfield AR**, Tobias R, Terblanche J, Girdwood AH, Fataar S, Harries-Jones R, Kernoff L, Marks IN. Preoperative external biliary drainage in obstructive jaundice. A prospective controlled clinical trial. *Lancet* 1982; **2**: 896-899 [PMID: 6126752]

2   **Sewnath ME**, Karsten TM, Prins MH, Rauws EJ, Obertop H, Gouma DJ. A meta-analysis on the efficacy of preoperative biliary drainage for tumors causing obstructive jaundice. *Ann Surg* 2002; **236**: 17-27

3   **van der Gaag NA**, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* 2010; **362**: 129-137 [PMID: 20071702 DOI: 10.1056/NEJMoa0903230]

4   **Cotton PB**, Lehman G, Vennes J, Geenen JE, Russell RC, Meyers WC, Liguory C, Nickl N. Endoscopic sphincterotomy complications and their management: an attempt at consensus. *Gastrointest Endosc* 1991; **37**: 383-393 [PMID: 2070995]

5   **Bailey AA**, Bourke MJ, Williams SJ, Walsh PR, Murray MA, Lee EY, Kwan V, Lynch PM. A prospective randomized trial of cannulation technique in ERCP: effects on technical success and post-ERCP pancreatitis. *Endoscopy* 2008; **40**: 296-301 [PMID: 18389448 DOI: 10.1055/s-2007-995566]

6   **Qiu YD**, Bai JL, Xu FG, Ding YT. Effect of preoperative biliary drainage on malignant obstructive jaundice: a meta-analysis. *World J Gastroenterol* 2011; **17**: 391-396 [PMID: 21253401 DOI: 10.3748/wjg.v17.i3.391]

7   **Fang Y**, Gurusamy KS, Wang Q, Davidson BR, Lin H, Xie X, Wang C. Meta-analysis of randomized clinical trials on safety and efficacy of biliary drainage before surgery for obstructive jaundice. *Br J Surg* 2013; **100**: 1589-1596 [PMID: 24264780 DOI: 10.1002/bjs.9260]

8   **Sun C**, Yan G, Li Z, Tzeng CM. A meta-analysis of the effect of preoperative biliary stenting on patients with obstructive jaundice. *Medicine* (Baltimore) 2014; **93**: e189 [PMID: 25474436 DOI: 10.1097/MD.0000000000000189]

9   **Velanovich V**, Kheibek T, Khan M. Relationship of postoperative complications from preoperative biliary stents after pancreaticoduodenectomy. A new cohort analysis and meta-analysis of modern studies. *JOP* 2009; **10**: 24-29 [PMID: 19129611]

10 **Jinkins LJ**, Parmar AD, Han Y, Duncan CB, Sheffield KM, Brown KM, Riall TS. Current trends in preoperative biliary stenting in patients with pancreatic cancer. *Surgery* 2013; **154**: 179-189 [PMID: 23889947 DOI: 10.1016/j.surg.2013.03.016]

11 **Cortes A**, Sauvanet A, Bert F, Janny S, Sockeel P, Kianmanesh R, Ponsot P, Ruszniewski P, Belghiti J. Effect of bile contamination on immediate outcomes after pancreaticoduodenectomy for tumor. *J Am Coll Surg* 2006; **202**: 93-99 [PMID: 16377502]

12 **Smith RA**, Dajani K, Dodd S, Whelan P, Raraty M, Sutton R, Campbell F, Neoptolemos JP, Ghaneh P. Preoperative resolution of jaundice following biliary stenting predicts more favourable early survival in resected pancreatic ductal adenocarcinoma. *Ann Surg Oncol* 2008; **15**: 3138-3146 [PMID: 18787902 DOI: 10.1245/s10434-008-0148-z]

13 **Morris-Stiff G**, Tamijmarane A, Tan YM, Shapey I, Bhati C, Mayer AD, Buckels JA, Bramhall SR, Mirza DF. Pre-operative stenting is associated with a higher prevalence of post-operative complications following pancreatoduodenectomy. *Int J Surg* 2011; **9**: 145-149 [PMID: 21029795 DOI: 10.1016/j.ijsu.2010.10.008]

14 **Isla AM**, Griniatsos J, Riaz A, Karvounis E, Williamson RC. Pancreaticoduodenectomy for periampullary malignancies: the effect of bile colonization on the postoperative outcome. *Langenbecks Arch Surg* 2007; **392**: 67-73 [PMID: 17089176]

15 **Roque J**, Ho SH, Goh KL. Preoperative drainage for malignant biliary strictures: is it time for self-expanding metallic stents? *Clin Endosc* 2015; **48**: 8-14 [PMID: 25674520 DOI: 10.5946/ce.2015.48.1.8]

16 **Lai EC**, Lau SH, Lau WY. The current status of preoperative biliary drainage for patients who receive pancreaticoduodenectomy for periampullary carcinoma: a comprehensive review. *Surgeon* 2014; **12**: 290-296 [PMID: 24650759 DOI: 10.1016/j.surge.2014.02.004]

17 **Povoski SP**, Karpeh MS, Conlon KC, Blumgart LH, Brennan MF. Association of preoperative biliary drainage with postoperative outcome following pancreaticoduodenectomy. *Ann Surg* 1999; **230**: 131-142 [PMID: 10450725]

18 **Sung JY**, Leung JW, Shaffer EA, Lam K, Olson ME, Costerton JW. Ascending infection of the biliary tract after surgical sphincterotomy and biliary stenting. *J Gastroenterol Hepatol* 1992; **7**: 240-245 [PMID: 1611012]

19 **Limongelli P**, Pai M, Bansi D, Thiallinagram A, Tait P, Jackson J, Habib NA, Williamson RC, Jiao LR. Correlation between preoperative biliary drainage, bile duct contamination, and postoperative outcomes for pancreatic surgery. *Surgery* 2007; **142**: 313-318 [PMID: 17723881]

20 **Lermite E**, Pessaux P, Teyssedou C, Etienne S, Brehant O, Arnaud JP. Effect of preoperative endoscopic biliary drainage on infectious morbidity after pancreatoduodenectomy: a case-control study. *Am J Surg* 2008; **195**: 442-446 [PMID: 18304506 DOI: 10.1016/j.amjsurg.2007.03.016]

21 **Mezhir JJ**, Brennan MF, Baser RE, D'Angelica MI, Fong Y, DeMatteo RP, Jarnagin WR, Allen PJ. A matched case-control study of preoperative biliary drainage in patients with pancreatic adenocarcinoma: routine drainage is not justified. *J Gastrointest Surg* 2009; **13**: 2163-2169 [PMID: 19774424 DOI: 10.1007/s11605-009-1046-9]

22 **Garcea G**, Chee W, Ong SL, Maddern GJ. Preoperative biliary drainage for distal obstruction: the case against revisited. *Pancreas* 2010; **39**: 119-126 [PMID: 19940799 DOI: 10.1097/MPA.0b013e3181bd65de]

23 **Ngu W**, Jones M, Neal CP, Dennison AR, Metcalfe MS, Garcea G. Preoperative biliary drainage for distal biliary obstruction and post-operative infectious complications. *ANZ J Surg* 2013; **83**: 280-286 [PMID: 23043467 DOI: 10.1111/j.1445-2197.2012.06296.x]

24 **Kitahata Y**, Kawai M, Tani M, Hirono S, Okada K, Miyazawa M, Shimizu A, Yamaue H. Preoperative cholangitis during biliary drainage increases the incidence of postoperative severe complications after pancreaticoduodenectomy. *Am J Surg* 2014; **208**: 1-10 [PMID: 24530042 DOI: 10.1016/j.amjsurg.2013.10.021]

25 **Fang Y**, Gurusamy KS, Wang Q, Davidson BR, Lin H, Xie X, Wang C. Pre-operative biliary drainage for obstructive jaundice. *Cochrane Database Syst Rev* 2012; **9**: CD005444 [DOI: 10.1002/14651858.CD005444.pub3]

26 **Jagannath P**, Dhir V, Shrikhande S, Shah RC, Mullerpatan P, Mohandas KM. Effect of preoperative biliary stenting on immediate outcome after pancreaticoduodenectomy. *Br J Surg* 2005; **92**: 356-361 [PMID: 15672425]

27 **Coates JM**, Beal SH, Russo JE, Vanderveen KA, Chen SL, Bold RJ, Canter RJ. Negligible effect of selective preoperative biliary drainage on perioperative resuscitation, morbidity, and mortality in patients undergoing pancreaticoduodenectomy. *Arch Surg* 2009; **144**: 841-847 [PMID: 19797109 DOI: 10.1001/archsurg.2009.152]

28 **Pitt HA**, Gomes AS, Lois JF, Mann LL, Deutsch LS, Longmire WP. Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? *Ann Surg* 1985; **201**: 545-553 [PMID: 2986562]

29 **Hong SK**, Jang JY, Kang MJ, Han IW, Kim SW. Comparison of clinical outcome and cost-effectiveness after various preoperative biliary drainage methods in periampullary cancer with obstructive jaundice. *J Korean Med Sci* 2012; **27**: 356-362 [PMID: 22468097 DOI: 10.3346/jkms.2012.27.4.356]

30   **Strom TJ**, Klapman JB, Springett GM, Meredith KL, Hoffe SE, Choi J, Hodul P, Malafa MP, Shridhar R. Comparative long-term outcomes of upfront resected pancreatic cancer after preoperative biliary drainage. *Surg Endosc* 2015; Epub ahead of print [PMID: 25631110]

31   **Murakami Y**, Uemura K, Hashimoto Y, Kondo N, Nakagawa N, Sasaki H, Hatano N, Kohmo T, Sueda T. Does preoperative biliary drainage compromise the long-term survival of patients with pancreatic head carcinoma? *J Surg Oncol* 2015; **111**: 270-276 [PMID: 25266938 DOI: 10.1002/jso.23797]

32   **Sugiyama H**, Tsuyuguchi T, Sakai Y, Nisikawa T, Miyazaki M, Yokosuka O. Preoperative drainage for distal biliary obstruction: endoscopic stenting or nasobiliary drainage? *Hepatogastroenterology* 2013; **60**: 231-234 [PMID: 22975651]

33   **Park SY**, Park CH, Cho SB, Lee WS, Kim JC, Cho CK, Joo YE, Kim HS, Choi SK, Rew JS. What is appropriate procedure for preoperative biliary drainage in patients with obstructive jaundice awaiting pancreaticoduodenectomy? *Surg Laparosc Endosc Percutan Tech* 2011; **21**: 344-348 [PMID: 22002271 DOI: 10.1097/SLE.0b013e3182318d2f]

34   **Singal AK**, Ross WA, Guturu P, Varadhachary GR, Javle M, Jaganmohan SR, Raju RP, Fleming JB, Raju GS, Kuo YF, Lee JH. Self-expanding metal stents for biliary drainage in patients with resectable pancreatic cancer: single-center experience with 79 cases. *Dig Dis Sci* 2011; **56**: 3678-3684 [PMID: 21750930 DOI: 10.1007/s10620-011-1815-7]

35   **Decker C**, Christein JD, Phadnis MA, Wilcox CM, Varadarajulu S. Biliary metal stents are superior to plastic stents for preoperative biliary decompression in pancreatic cancer. *Surg Endosc* 2011; **25**: 2364-2367 [PMID: 21373939 DOI: 10.1007/s00464-010-1552-6]

36   **Cavell LK**, Allen PJ, Vinoya C, Eaton AA, Gonen M, Gerdes H, Mendelsohn RB, D'Angelica MI, Kingham TP, Fong Y, Dematteo R, Jarnagin WR, Kurtz RC, Schattner MA. Biliary self-expandable metal stents do not adversely affect pancreaticoduodenectomy. *Am J Gastroenterol* 2013; **108**: 1168-1173 [PMID: 23545711 DOI: 10.1038/ajg.2013.93]

37   **Haapamäki C**, Seppänen H, Udd M, Juuti A, Halttunen J, Kiviluoto T, Sirén J, Mustonen H, Kylänpää L. Preoperative biliary decompression preceding pancreaticoduodenectomy with plastic or self-expandable metallic stent. *Scand J Surg* 2015; **104**: 79-85 [PMID: 25028410]

38   **Kubota K**, Sato T, Watanabe S, Hosono K, Kobayashi N, Mori R, Taniguchi K, Matsuyama R, Endo I, Nakajima A. Covered self-expandable metal stent deployment promises safe neoadjuvant chemoradiation therapy in patients with borderline resectable pancreatic head cancer. *Dig Endosc* 2014; **26**: 77-86 [PMID: 23551230 DOI: 10.1111/den.12049]

39   **Siddiqui AA**, Mehendiratta V, Loren D, Kowalski T, Fang J, Hilden K, Adler DG. Self-expanding metal stents (SEMS) for preoperative biliary decompression in patients with resectable and borderline-resectable pancreatic cancer: outcomes in 241 patients. *Dig Dis Sci* 2013; **58**: 1744-1750 [PMID: 23179157 DOI: 10.1007/s10620-012-2482-z]

40   **Kaneko T**, Sugimori K, Shimizu Y, Miwa H, Kameta E, Koh R, Numata K, Tanaka K, Maeda S. Efficacy of plastic stent placement inside bile ducts for the treatment of unresectable malignant hilar obstruction (with videos). *J Hepatobiliary Pancreat Sci* 2014; **21**: 349-355 [PMID: 24123806 DOI: 10.1002/jhbp.41]

41   **Ishiwatari H**, Hayashi T, Ono M, Sato T, Kato J. Newly designed plastic stent for endoscopic placement above the sphincter of Oddi in patients with malignant hilar biliary obstruction. *Dig Endosc* 2013; **25** Suppl 2: 94-99 [PMID: 23617658 DOI: 10.1111/den.12080]

42   **Kobayashi N**, Watanabe S, Hosono K, Kubota K, Nakajima A, Kaneko T, Sugimori K, Tokuhisa M, Goto A, Mori R, Taniguchi K, Matsuyama R, Endo I, Maeda S, Ichikawa Y. Endoscopic inside stent placement is suitable as a bridging treatment for preoperative biliary tract cancer. *BMC Gastroenterol* 2015; **15**: 8 [PMID: 25649526 DOI: 10.1186/s12876-015-0233-2]

43   **Rerknimitr R**, Kullavanijaya P. Operable malignant jaundice: To stent or not to stent before the operation? *World J Gastrointest Endosc* 2010; **2**: 10-14 [PMID: 21160672 DOI: 10.4253/wjge.v2.i1.10]

44   **Martignoni ME**, Wagner M, Krähenbühl L, Redaelli CA, Friess H, Büchler MW. Effect of preoperative biliary drainage on surgical outcome after pancreatoduodenectomy. *Am J Surg* 2001; **181**: 52-59; discussion 87 [PMID: 11248177]

45   **Sewnath ME**, Birjmohun RS, Rauws EA, Huibregtse K, Obertop H, Gouma DJ. The effect of preoperative biliary drainage on postoperative complications after pancreaticoduodenectomy. *J Am Coll Surg* 2001; **192**: 726-734 [PMID: 11400966]

46   **Hodul P**, Creech S, Pickleman J, Aranha GV. The effect of preoperative biliary stenting on postoperative complications after pancreaticoduodenectomy. *Am J Surg* 2003; **186**: 420-425 [PMID: 14599600]

47   **Barnett SA**, Collier NA. Pancreaticoduodenectomy: does preoperative biliary drainage, method of pancreatic reconstruction or age influence perioperative outcome? A retrospective study of 104 consecutive cases. *ANZ J Surg* 2006; **76**: 563-568 [PMID: 16813619]

48   **Singhirunnusorn J**, Roger L, Chopin-Laly X, Lepilliez V, Ponchon T, Adham M. Value of preoperative biliary drainage in a consecutive series of resectable periampullary lesions. From randomized studies to real medical practice. *Langenbecks Arch Surg* 2013; **398**: 295-302 [PMID: 23007383 DOI: 10.1007/s00423-012-1000-2]

49   **Sudo T**, Murakami Y, Uemura K, Hayashidani Y, Hashimoto Y, Ohge H, Sueda T. Specific antibiotic prophylaxis based on bile cultures is required to prevent postoperative infectious complications in pancreatoduodenectomy patients who have undergone preoperative biliary drainage. *World J Surg* 2007; **31**: 2230-2235 [PMID: 17726628]

50   **Son JH**, Kim J, Lee SH, Hwang JH, Ryu JK, Kim YT, Yoon YB, Jang JY, Kim SW, Cho JY, Yoon YS, Han HS, Woo SM, Lee WJ, Park SJ. The optimal duration of preoperative biliary drainage for periampullary tumors that cause severe obstructive jaundice. *Am J Surg* 2013; **206**: 40-46 [PMID: 23706545 DOI: 10.1016/j.amjsurg.2012.07.047]

ORIGINAL ARTICLE

Observational Study

Virological response and resistance mutations to NS3/4A inhibitors in hepatitis C virus-human immunodeficiency virus coinfection

Alissa Naqvi, Valérie Giordanengo, Brigitte Dunais, Francine de Salvador-Guillouet, Isabelle Perbost, Jacques Durant, Pascal Pugliese, Aline Joulié, Pierre Marie Roger, Eric Rosenthal

Alissa Naqvi, Brigitte Dunais, Francine de Salvador-Guillouet, Isabelle Perbost, Jacques Durant, Pascal Pugliese, Pierre Marie Roger, Infectious Diseases Department, Archet Hospital, Centre Hospitalier Universitaire, 06200 Nice, France

Valérie Giordanengo, Virology Laboratory, Archet Hospital, Centre Hospitalier Universitaire, 06200 Nice, France

Aline Joulié, Eric Rosenthal, Internal Medicine Department, Archet Hospital, Centre Hospitalier Universitaire, 06200 Nice, France

Correspondence to: Eric Rosenthal, Professor, Internal Medicine Department, Archet Hospital, Centre Hospitalier Universitaire, 51 Route de Saint-Antoine, 06200 Nice, France. rosenthal.e@chu-nice.fr
Telephone: +33-4-92035861

Abstract

AIM: To evaluate virological response to telaprevir or boceprevir in combination with pegylated interferon and ribavirin and resistance mutations to NS3/4A inhibitors in hepatitis C virus-human immunodeficiency virus (HCV-HIV) coinfected patients in a real life setting.

METHODS: Patients with HCV genotype 1-HIV coinfection followed in Nice University Hospital internal medicine and infectious diseases departments who initiated treatment including pegylated interferon and ribavirin (PegIFN/RBV) + telaprevir or boceprevir, according to standard treatment protocols, between August 2011 and October 2013 entered this observational study. Patient data were extracted from an electronic database (Nadis®). Liver fibrosis was measured by elastometry (Fibroscan®) with the following cut-off

values: F0-F1: < 7.1 kPa, F2: 7.1-9.5 kPa, F3: 9.5-14.5 kPa, F4: ≥ 14.5 kPa. The proportion of patients with sustained virological response (SVR) twelve weeks after completing treatment, frequency and type of adverse events, and NS3/4A protease inhibitor mutations were described.

RESULTS: Forty-one patients were included: 13 (31.7%) patients were HCV-treatment naïve, 22 (53.7%) had advanced liver fibrosis or cirrhosis (Fibroscan stage F3 and F4); none had decompensated cirrhosis or hepatocellular carcinoma; all were receiving antiretroviral treatment, consisting for most them (83%) in either a nucleoside reverse-transcriptase inhibitor/protease inhibitor or/integrase inhibitor combination; all patients had undetectable HIV-RNA. One patient was lost to follow-up. SVR was achieved by 52.5% of patients. Five patients experienced virological failure during treatment and four relapsed. Seven discontinued treatment due to adverse events. Main adverse events included severe anemia (88%) and rash (25%). NS3/4A protease mutations were analyzed at baseline and at the time of virological failure in the 9 patients experiencing non-response, breakthrough or relapse. No baseline resistance mutation could predict resistance to HCV protease inhibitor-based treatment.

CONCLUSION: Telaprevir and boceprevir retain their place among potential treatment strategies in HIV-HCV coinfected patients including those with advanced compensated liver disease and who failed previous PegIFN/RBV therapy.

Key words: Telaprevir; Boceprevir; Hepatitis C virus-human immunodeficiency virus coinfection; Resistance mutations

Core tip: Data regarding treatment of hepatitis C virus (HCV) infection with triple combination regimen including interferon/ribavirin and a protease inhibitor (telaprevir or boceprevir) among difficult to treat human immunodeficiency virus (HIV)-HCV co-infected patients are lacking. Most of the patients included in this single-center observational study had already failed a previous dual treatment course and had severe liver fibrosis, one out of six being both cirrhotic and non-responder to prior therapy. More than one of two patients displayed sustained virological response, suggesting that in low-income countries, telaprevir and boceprevir may retain their place among potential treatment strategies in HIV-HCV coinfected patients.

INTRODUCTION

Therapeutic resources against hepatitis C infection are currently expanding, with remarkable success rates compared to previous results with dual pegylated interferon + ribavirin (PegIFN/RBV) regimens[1]. However some of these new compounds can be associated with considerable costs making them unaffordable in low resource settings or among uninsured patients with low incomes. The situation can be further complicated by human immunodeficiency virus (HIV) co-infection, requiring the adjunction of antiretroviral treatment. The availability of multiple approaches for the management of hepatitis C virus (HCV) in HIV-HCV co-infection thus provides a choice of strategies covering a range of costs while offering the patient reasonable chances of therapeutic success.

Most data concerning treatment of HCV infection among HIV-HCV co-infected patients have mainly been based on clinical trials[2-5]. Few have included cirrhotic patients or non-responders to prior HCV therapy with PegIFN/RBV combination, and none to our knowledge have concerned patients who were both cirrhotic and non-responders. We report the virological response to triple combination therapy including IFN/RBV and a protease inhibitor (telaprevir or boceprevir) among a cohort of HIV-HCV genotype 1 co-infected patients followed in a University Hospital.

MATERIALS AND METHODS

This was an observational single-center study concerning all genotype 1 HCV-HIV co-infected patients followed in Nice University Hospital internal medicine and infectious diseases departments who initiated treatment including PegIFN/RBV + telaprevir or boceprevir between August 2011 and October 2013 and who were not participating in a clinical trial.

Liver fibrosis was measured by elastometry (Fibroscan®) with the following cut-off values: F0-F1: < 7.1 kPa, F2: 7.1-9.5 kPa, F3: 9.5-14.5 kPa, F4: ≥ 14.5 kPa. Cirrhosis was considered present for values above 14.5 kPa prior to HCV treatment initiation. Virological response to treatment was assessed at 4, 12, 24 and 48 wk. Rapid virological response (RVR) was defined as undetectable HCV-RNA 4 wk after treatment initiation, and sustained virological response (SVR) as undetectable HCV-RNA 12 wk following completion of HCV treatment. Persistently detectable HCV-RNA during treatment was considered as non-response to treatment, while treatment breakthrough concerned patients in whom an initially positive response was followed by renewed detectable HCV-RNA during treatment. Relapse was defined as recurrence of viraemia in patients whose viral load had become undetectable at the end of treatment. Eventual exposure and response to a previous dual

Table 1 Primers specific for the hepatitis C virus NS3/4A protease gene sequence used for polymerase chain reaction

HCV genotype 1	Primers	Sequences 5'-3'	H77 location
	G1F1	CTB CTS GGR CCR GCC GAT	3372-3390
	G1R1	CCA CYT GGW AKS TCT GSG G	3998-4016
	MarsF3	ACS GCR GCR TGY GGG GAC AT	3309-3328
	MarsR2	GTG CTC TTR CCG CTR CCR GT	4035-4054

Genotyping: S = G or C; R = A or G; Y = C or T; W = A or T; K = G or T. HCV: Hepatitis C virus.

PegIFN/RBV regimen was investigated. Patients for whom no HCV viral load was available at 12 wk following dual treatment initiation could not be assessed for prior treatment response. Premature treatment discontinuation of previous dual therapy that was considered related to adverse events was differentiated from virological failure.

Patient data were extracted from an electronic database (Nadis®)[6] and included date of initiation and end of anti-HCV treatment, dose of RBV, peg-interferon, boceprevir and telaprevir, response to previous anti-HCV treatment, HIV-RNA, HCV genotype, HCV-RNA at each stage of follow-up (week 4, 12, 24, 48, and at least 12 wk after completing treatment), antiretroviral treatment, and CD4 T-cell count. The following adverse events were recorded: grade 2 to 4 anemia [hemoglobin (Hb) < 9 g/dL], leucopenia, thrombocytopenia, severe infection, decompensated cirrhosis, rashes, as well as death and cause thereof. erythropoietin (EPO) use and blood transfusions were also reported.

NS3/4A genotyping
In order to genotype HCV strains, total nucleic acids were extracted from 500 μL of plasma with the NucliSENS® easyMAG® automated platform (BioMerieux). The NS3/4A protease sequence (745 nucleotides) was amplified by reverse-transcriptase nested polymerase chain reaction with protocol and primers described elsewhere[7] (Table 1) and evaluated in a multicenter quality control study[8].

Briefly, 40 μL of reaction mixture contained 2X Super Script Ⅲ One-Step reaction buffer with 0.4 mmol/L dNTP and 5 mmol/L MgSO₄, 0.2 μmol/L of each sense and anti-sense primers, 1 μL of Super Script Ⅲ RT/Platinium Taq High Fidelity and 10 μL of RNA extract. Amplification was performed using Biometra thermocycler: 30 min at 55 ℃ followed by 2 min at 94 ℃, 40 cycles with 30 s at 94 ℃, 1 min at 59 ℃, 1 min at 68 ℃, and a final extension step at 68 ℃.

When needed, a nested polymerase chain reaction (PCR) was performed involving the inner genotype-specific primers in 40 μL of reaction mixture contained 10X Invitrogen Thermal ace reaction buffer with 0.5 mmol/L dNTP, 0.2 μmol/L of each sense and anti-sense primers and 10 μL of purified product of first step PCR using: 2 min at 95 ℃, 35 cycles with 30 s at 95 ℃, 30 s at 59 ℃, 1 min at 74 ℃, and a final extension step at 74 ℃.

PCR-amplified DNA was purified, genotype-specific primers for the inner PCR were used for bidirectional sequencing and automated dideoxynucleotide termination sequencing was performed with BigDye Terminator using a 3130XL Genetic Analyzer (Applied Biosystems) then compared to the HCV-H77 reference strain using Sequence Navigator software™ (Applied Biosystems) and Geno2pheno.

Statistical analysis
Variables at treatment initiation were expressed as frequencies (percentages) for categorical variables, or median (interquartile range) for continuous variables. The rate of treatment response was calculated as the number of patients with undetectable HCV viral load divided by the number of patients with available data on HCV-RNA viral load at the considered visit.

In case of early treatment discontinuation with a detectable HCV viral load, the patient was considered as failing treatment at each subsequent visit up to week 48. In case of early treatment discontinuation with undetectable HCV viral load, the subsequent HCV viral load measurements were used to assess treatment efficacy.

Data analysis was performed using Epi-Info™ version 7 software.

RESULTS
Patient characteristics at treatment initiation are described in Table 2.

Among 41 patients included in the study, 13/41 (31.7%) were HCV-treatment naïve. Twenty-two patients (53.7%) had advanced liver fibrosis or cirrhosis (Fibroscan stage F3 and F4). None had decompensated cirrhosis or hepatocellular carcinoma. All were receiving antiretroviral treatment, consisting for most patients (83%) in either a nucleoside reverse-transcriptase inhibitor/protease inhibitor or/integrase inhibitor combination (Figure 1). All had undetectable HIV-RNA. Thirty-seven patients received telaprevir and 4 were treated with boceprevir in addition to IFN/RBV combination therapy. One patient was lost to follow-up.

The number of patients with undetectable HCV viral load at different stages of the treatment course is shown in Figure 2. Twenty-seven patients (67.5%)
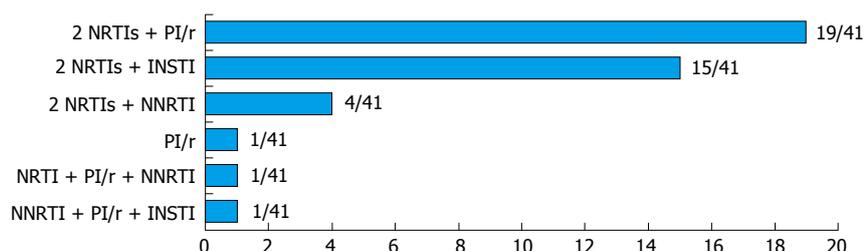
Figure 1 Antiretroviral treatment regimens at initiation of triple anti-hepatitis C virus therapy (*n* = 41). PI: Protease inhibitor (atazanavir, darunavir, lopinavir, ritonavir boost); NRTI: Nucleoside analogue reverse transcriptase inhibitor (tenofovir, emtricitabine, abacavir, lamivudine); NNRTI: Non-nucleoside analogue reverse transcriptase inhibitor (efavirenz, etravirine); INSTI: Integrase strand transfer inhibitor (raltegravir).

Table 2 Patient characteristics at initiation of triple anti-hepatitis C virus therapy (*n* = 41)	
Age: median (IQR)	51 (48-55)
Male gender	35 (85.4%)
Genotype	
1a	32 (78.0%)
1b	9 (22.0%)
HCV treatment-naïve	13 (31.7%)
Prior HCV-treatment response	
Non-responders	14 (34.1%)
Breakthrough	1 (2.5%)
Relapse	5 (12.2%)
Premature treatment discontinuation	3 (4.9%)
Missing data	5 (14.6%)
Log HCV-RNA	5.8 (5.3-6.1)
Fibrosis stage	
F0-F1	12 (29.3%)
F2	7 (17.0%)
F3	5 (12.2%)
F4	17 (41.5%)
CD4 T-cells/mm^3: median (IQR)	540 (441-782)
HIV-RNA < 40 copies/mL	41 (100.0%)

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IQR: Interquartile range.

| Table 3 Main adverse events in 40 patients with available follow-up	
Rash	10 (25%)
Anemia Hb < 13 g/dL (men), < 12 g/dL (women)	39 (98%)
Severe anemia (< 9 g/dL or decrease > 3 g/dL)	35 (88%)
Ribavirin dose decreased	23 (58%)
EPO administration	23 (58%)
Blood transfusion	2 (5%)

EPO: Erythropoietin; Hb: Hemoglobin.

Figure 2 Number of patients with undetectable hepatitis C virus-RNA between hepatitis C virus treatment initiation and sustained virological response 12. HCV: Hepatitis C virus; SVR: Sustained virological response.

(52.4%) exhibited SVR.

Fifteen patients did not complete the treatment course: 5 due to virological failure (3 viral breakthroughs, 2 non- or partial responders), 3 on patient's decision, and 7 because of adverse events. Eight patients did so within the first 12 wk, three between 12 and 24 wk, and four between 24 and 48 wk. Two of these patients were switched to a sofosbuvir/RBV regimen.

Four patients relapsed: 2 were HCV treatment-naïve with liver fibrosis stages F2 and F3, while the other 2 patients were treatment-experienced with a fibrosis score of F4. Among the seven previously non-responding cirrhotic patients, 2 achieved SVR, 1 relapsed, 1 experienced virological breakthrough, 1 did not respond and 2 discontinued treatment due to adverse events (gingival haemorrhage and psychiatric decompensation).

Adverse events

Among the 15 patients who discontinued treatment prematurely, seven developed the following adverse events: flare-up of porphyria cutanea tarda (1), gingival haemorrhage (1), psychiatric disorder (1), thrombocytopenia (1), fatigue (1), cirrhotic decompensation (1) and acute pancreatitis (1) (Table 3).

Thirty five (85%) patients developed severe anemia (Hb < 9 g/dL) requiring either administration of EPO, blood transfusion or both (Table 3).

NS3/4A protease genotyping

Pre- and post treatment genotyping of the NS3 protease was performed using standard Sanger sequencing for

displayed RVR. Response to treatment according to HCV treatment history, stage of fibrosis and HCV genotype is described in Figure 3 among the 40 patients with available follow-up. Twenty-one patients (52.5%) displayed SVR: 6 were treatment-naïve, 5 were partial or non-responders to prior dual agent HCV-treatment regimens, 3 had relapsed, 3 had discontinued treatment prematurely, and response to initial treatment was unspecified for 4 patients. Therapeutic success was observed among all categories except in the patient who had previously experienced virological breakthrough. Among patients with advanced fibrosis (F3-F4), 11/21

Figure 3 Sustained virological response at 12 wk following treatment discontinuation according to prior treatment history, fibrosis severity and genotype in 40 patients with available follow-up. PTI: Premature treatment interruption; HCV: Hepatitis C virus.

Table 4 Baseline and post treatment NS3/4A-mutations among patients failing hepatitis C virus treatment

Genotype	Previously treated	HCV PI	Response to treatment	HIV PI	Pre-treatment HCV VL	Baseline NS3/4A-mutations	Baseline fold-change	Post-treatment NS3/4A-mutations	Post-treatment fold change[1]
1a	Yes	Tela	Non-responder	Atazanavir	5.9	0		V36M, R155K	62
1a	Yes	Tela	Non-responder	0	5.8	0		V36M, R155K	62
1a	Yes	Tela	Breakthrough	Atazanavir	6.0	0		R155K	7.4
1a	Yes	Tela	Breakthrough	Atazanavir	6.5	0		V36M	6.8-10.0
1a	No	Tela	Breakthrough	Atazanavir	6.0	0		V36M, R155K	62
1b	Yes	Tela	Relapse	Atazanavir	6.4	I132V	1.8	I132V, V36A	7.4-7.5
1a	No	Tela	Relapse	0	6.3	I132V	1.8	I132V, V36A	7.4-7.5
1b	No	Tela	Relapse	0	4.9	I132V	1.8	I132V, V36A	7.4-7.5
1a	NA	Boce	Relapse	Darunavir	NA	NA	NA	T54S, R155K	8.5

[1]Fold-change in EC_{50} with regard to specific PI received. HCV: Hepatitis C virus; Tela: Telaprevir; Boce: Boceprevir; HIV PI: Human immunodeficiency virus protease inhibitor; NA: Data unavailable; VL: Viral load.

the nine patients who experienced treatment failure (3 viral breakthroughs, 2 non- or partial responders, and 4 relapses). Their NS3/4A sequences were compared to the HCV-H77 reference strain (reference sequence AF009606) using Sequence Navigator software™ (Applied Biosystems) and were analysed with Geno2-pheno HCV. Both methods yielded the same resistance mutations. Details of NS3/4A amino acid resistance mutations are displayed in Table 4. Pre-treatment plasma samples were available for 3 of the 4 relapsing patients, showing an *I132V* mutation conveying possible resistance to telaprevir. All three were treated with telaprevir and displayed V36A mutations following exposure, conferring 7.4-7.5 fold changes in EC_{50}. The fourth patient, who was the only one to have received boceprevir among patients who failed treatment, had T54S and R155K substitutions following exposure. The non- and partial responders both had no initial mutations but developed V36M and R155K conferring a 62-fold change in antiviral activity.

No mutations were initially observed among patients who developed virological breakthrough, who all displayed R155K, V36M, or both substitutions after exposure, the latter combination associated with a 62-fold change in EC_{50}.

DISCUSSION

This observational study assessed the effectiveness of a PegIFN/RBV + HCV-protease inhibitor combination in difficult-to-treat HIV co-infected patients most of whom had already failed a previous dual treatment course and had severe liver fibrosis (54%), one out of six patients being both cirrhotic and non-responder to prior therapy. In spite of such adverse circumstances, among the 40 patients that could be assessed, 52.5% displayed SVR, and one out of three among cirrhotic, non-responders to previous therapy.

Two open-label, single-arm, phase 2 clinical trials (ANRS HC26 TelapreVIH and HC27 BocepreVIH) recently investigated the effectiveness of PegIFN + RBV + protease inhibitor combination in pre-treated patients[4,5]. In the present study performed in a "real-life" setting, the SVR rate was similar to that obtained in the BocepreVIH trial (53%) but lower than that in the TelapreVIH trial (80%) while the proportion of

cirrhotic patients (41.5%) was higher (TelapreVIH 23%, BocepreVIH 17%) and patients that were both cirrhotic and non-responders were not included in those trials. A comparison between these two agents was not possible in the present study due to the small number of patients receiving boceprevir. Lacombe *et al*[9] reported favorable results of triple therapy including telaprevir in 20 HCV genotype 1 mostly cirrhotic HIV-coinfected patients who had failed PegIFN/RBV treatment with 55% success rate at week 24. Overall treatment safety in our patient cohort was comparable to that observed in the above-mentioned clinical trials[4,5]. One out of four patients discontinued treatment due to adverse events or by choice. Anemia was often severe and was the most frequent adverse effect (88%), requiring EPO administration in 60% of patients, without resulting in treatment discontinuation.

In the present study, an analysis of viral populations following treatment failure was performed by sequencing method and sequences were aligned with the NS3/4A sequence from the HCV genotype 1a H77 strain. All nine patients with virological failure displayed a *V36M/R155K* mutation. This was more frequent for subtype 1a. No amino-acid resistance mutation before treatment was found in these patients using standard Sanger sequencing, so that the emergence of telaprevir-resistant variants could not be predicted from baseline findings. On the other hand, non-response to treatment may be explained by the subtype-specific resistant variants generated by *de novo* reverse mutation after treatment failure and the relatively higher fitness of these variants, notably R155K[10], or by the presence of minority resistant variants which should be detected by deep-sequencing[11]. In a recent paper, Aherfi *et al*[12] describe drug concentrations and NS3/4A protease genotyping during therapy with these two agents and report naturally occurring variants with decreased susceptibility to HCV-protease inhibitors (PIs) at baseline in 20% of their cohort of 30 genotype 1 HCV-infected patients; out of 7 treatment failures, six patients displayed amino acid substitutions associated with decreased susceptibility to PIs.

Experience acquired through HIV antiretroviral therapy shows that antiviral resistance, treatment compliance issues and the need for customized treatment regimens require availability of a range of compounds to meet the specific circumstances of each individual patient. Our results suggest that these triple agent regimens including telaprevir or boceprevir should remain part of the HCV treatment arsenal for HIV co-infected patients, even among those with advanced compensated liver disease and who failed PegIFN/RBV therapy, providing patients do not present with baseline predictors of severe complications (platelet count > 100000/μL and serum albumin concentrations > 35 g/L)[13].

COMMENTS

Background
Over the last 15 years, the proportion of liver-related deaths in human immunodeficiency virus (HIV)-infected adults dramatically increased in developed countries, ranking first as a cause of mortality in HIV-hepatitis C virus (HCV)-infected individuals. This data makes treatment of chronic hepatitis C a priority in this population, including very difficult to treat patients.

Research frontiers
The addition of telaprevir and boceprevir to dual therapy with pegylated interferon and ribavirin (PegIFN/RBV) strongly improves the odds of achieving a sustained virological response in treatment-naïve HCV genotype 1 patients as well as in prior non-responders and relapsers when compared with standard therapy. Among HIV-HCV co-infected patients, data regarding treatment of HCV infection with triple combination regimen including IFN/RBV and telaprevir or boceprevir is scarce, more particularly in very difficult-to-treat patients with cirrhosis and/or prior null response to PegIFN/RBV therapy.

Innovations and breakthroughs
This observational study showed the effectiveness of a PegIFN/RBV + HCV-protease inhibitor combination in difficult-to-treat HIV coinfected patients, most of whom had already failed a previous dual treatment course and had severe liver fibrosis, one out of six patients being both cirrhotic and non-responder to prior therapy.

Applications
The cost of interferon-free regimens with very recent direct-acting antiviral drugs is prohibitive, making them inaccessible in many developing countries. This study suggests that in low-income countries, telaprevir and boceprevir may retain their place among potential treatment strategies in HIV-HCV coinfected patients including those with cirrhosis and/or prior null response to PegIFN/RBV therapy.

Terminology
Telaprevir and boceprevir are two NS3/4A protease inhibitors. These drugs were the first direct-acting antiviral agents approved by the United States Food and Drug Administration and the European Medicines Agency for the treatment of patients infected with HCV genotype 1.

Peer-review
This manuscript presented a clinical observation in evaluating the treatment with telaprevir or boceprevir in combination with pegylated interferon and ribavirin for HIV-HCV coinfected patients. This is a good topic.

REFERENCES

1 **Chastain CA**, Naggie S. Treatment of genotype 1 HCV infection in the HIV coinfected patient in 2014. *Curr HIV/AIDS Rep* 2013; **10**: 408-419 [PMID: 24272069 DOI: 10.1007/s11904-013-0182-8]

2 **Sulkowski M**, Pol S, Mallolas J, Fainboim H, Cooper C, Slim J, Rivero A, Mak C, Thompson S, Howe AY, Wenning L, Sklar P, Wahl J, Greaves W. Boceprevir versus placebo with pegylated interferon alfa-2b and ribavirin for treatment of hepatitis C virus genotype 1 in patients with HIV: a randomised, double-blind, controlled phase 2 trial. *Lancet Infect Dis* 2013; **13**: 597-605 [PMID: 23768747 DOI: 10.1016/S1473-3099(13)70149-X]

3 **Sulkowski MS**, Sherman KE, Dieterich DT, Bsharat M, Mahnke L, Rockstroh JK, Gharakhanian S, McCallister S, Henshaw J, Girard PM, Adiwijaya B, Garg V, Rubin RA, Adda N, Soriano V. Combination therapy with telaprevir for chronic hepatitis C virus genotype 1 infection in patients with HIV: a randomized trial. *Ann Intern Med* 2013; **159**: 86-96 [PMID: 23685940]

4 **Poizot-Martin** I, Bellissant E, Colson P, Renault A, Piroth L, Solas C, Bourlière M, Garraffo R, Halfon P, Molina JM. Boceprevir for previously treated HCV-HIV coinfected patients: the ANRS-HC27 BocepreVIH Trial. Atlanta: 21th CROI, 2014: Abstract 659LB

5 **Cotte L**, Braun J, Lascoux-Combe C, Vincent C, Valantin MA, Sogni P, Lacombe K, Neau D, Aumaitre H, Batisse D, de Truchis P, Gervais A, Michelet C, Morlat P, Vittecoq D, Rosa I, Bertucci I, Chevaliez S, Aboulker JP, Molina JM. Telaprevir for HIV/hepatitis C virus-coinfected patients failing treatment with pegylated interferon/ribavirin (ANRS HC26 TelapreVIH): an open-label,

single-arm, phase 2 trial. *Clin Infect Dis* 2014; **59**: 1768-1776 [PMID: 25139963 DOI: 10.1093/cid/ciu659]

6   **Pugliese P**, Cuzin L, Cabié A, Poizot-Martin I, Allavena C, Duvivier C, El Guedj M, de la Tribonnière X, Valantin MA, Dellamonica P. A large French prospective cohort of HIV-infected patients: the Nadis Cohort. *HIV Med* 2009; **10**: 504-511 [PMID: 19486189 DOI: 10.1111/hiv.12041]

7   **Vallet S**, Viron F, Henquell C, Le Guillou-Guillemette H, Lagathu G, Abravanel F, Trimoulet P, Soussan P, Schvoerer E, Rosenberg A, Gouriou S, Colson P, Izopet J, Payan C. NS3 protease polymorphism and natural resistance to protease inhibitors in French patients infected with HCV genotypes 1-5. *Antivir Ther* 2011; **16**: 1093-1102 [PMID: 22024525 DOI: 10.3851/IMP1900]

8   **Vallet S**, Larrat S, Laperche S, Le Guillou-Guillemette H, Legrand-Abravanel F, Bouchardeau F, Pivert A, Henquell C, Mirand A, André-Garnier E, Giordanengo V, Lagathu G, Thibault V, Scholtes C, Schvoerer E, Gaudy-Graffin C, Maylin S, Trimoulet P, Brochot E, Hantz S, Gozlan J, Roque-Afonso AM, Soussan P, Plantier JC, Charpentier C, Chevaliez S, Colson P, Mackiewicz V, Aguilera L, Rosec S, Gouriou S, Magnat N, Lunel-Fabiani F, Izopet J, Morand P, Payan C, Pawlotsky JM. Multicenter quality control of hepatitis C virus protease inhibitor resistance genotyping. *J Clin Microbiol* 2013; **51**: 1428-1433 [PMID: 23426922 DOI: 10.1128/JCM.03032-12]

9   **Lacombe K**, Valin N, Stitou H, Gozlan J, Thibault V, Boyd A, Poirier JM, Meynard JL, Valantin MA, Bottero J, Girard PM. Efficacy and tolerance of telaprevir in HIV-hepatitis C virus genotype 1-coinfected patients failing previous antihepatitis C virus therapy: 24-week results. *AIDS* 2013; **27**: 1356-1359 [PMID: 23925383 DOI: 10.1097/QAD.0b013e32836138d0]

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

11   **Akuta N**, Suzuki F, Seko Y, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Hara T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. Emergence of telaprevir-resistant variants detected by ultra-deep sequencing after triple therapy in patients infected with HCV genotype 1. *J Med Virol* 2013; **85**: 1028-1036 [PMID: 23588728 DOI: 10.1002/jmv.23579]

12   **Aherfi S**, Solas C, Motte A, Moreau J, Borentain P, Mokhtari S, Botta-Fridlund D, Dhiver C, Portal I, Ruiz JM, Ravaux I, Bregigeon S, Poizot-Martin I, Stein A, Gérolami R, Brouqui P, Tamalet C, Colson P. Hepatitis C virus NS3 protease genotyping and drug concentration determination during triple therapy with telaprevir or boceprevir for chronic infection with genotype 1 viruses, southeastern France. *J Med Virol* 2014; **86**: 1868-1876 [PMID: 25052594 DOI: 10.1002/jmv.24016]

13   **Hézode C**, Fontaine H, Dorival C, Larrey D, Zoulim F, Canva V, de Ledinghen V, Poynard T, Samuel D, Bourlière M, Zarski JP, Raabe JJ, Alric L, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Métivier S, Tran A, Serfaty L, Abergel A, Causse X, Di Martino V, Guyader D, Lucidarme D, Grando-Lemaire V, Hillon P, Feray C, Dao T, Cacoub P, Rosa I, Attali P, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP. Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC) - NCT01514890. *J Hepatol* 2013; **59**: 434-441 [PMID: 23669289 DOI: 10.1016/j.jhep.2013.04.035]