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EDITORIAL

Is there a role for adaptive immunity in nonalcoholic steatohepatitis?

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Abstract

The growing diffusion of nonalcoholic fatty liver disease (NAFLD) is a consequence of the worldwide increase in the prevalence of obesity. Oxidative stress is widely recognized to play a pivotal role in NAFLD evolution to nonalcoholic steatohepatitis (NASH). Here we review recent evidence suggesting that oxidative stress-derived antigens originating within fatty livers stimulate both humoral and cellular adaptive immune responses and the possible mechanisms involved in sustaining hepatic inflammation in NASH.

Key words: Liver; Adaptive immunity; Nonalcoholic steatohepatitis; Hepatic inflammation; Nonalcoholic fatty liver disease

Core tip: Nonalcoholic fatty liver disease (NAFLD) is becoming one of the most common hepatic diseases, yet the factors responsible for the wide inter-individual variability in NAFLD evolution to nonalcoholic steatohepatitis are still poorly understood. In this Editorial, we comment on recent evidence suggesting the involvement of adaptive immune responses in sustaining hepatic inflammation during NAFLD evolution.

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INTRODUCTION

One of the consequences of the epidemical worldwide diffusion of obesity is the growing prevalence of nonalcoholic fatty liver disease (NAFLD) that has become the most frequent hepatic lesion in western countries[1-3]. Although NAFLD is most often benign, in about 20%-30% of the patients steatosis evolves to nonalcoholic steatohepatitis (NASH) and fibrosis[1-3], making NAFLD an increasingly important cause of hepatic cirrhosis. In recent past, many studies have investigated the

pathogenesis of NAFLD/NASH[4]. However, several issues concerning the mechanisms responsible for promoting lobular inflammation are still incompletely understood. In particular, it is unclear why only a fraction of NAFLD patients develop steatohepatitis and what are the factors responsible for the large inter-individual variability in the disease evolution to fibrosis. Lymphocytes represent along with macrophages the most frequent inflammatory cells in lobular and periportal infiltrates of NASH[5]. This has several analogies with that observed in the adipose tissue of obese subjects where lymphocyte infiltration has emerged as an important player in orchestrating inflammation[6,7]. In fact, chemokines released by fat-resident macrophages recruit to the adipose tissue $CD4^+$/$CD8^+$ T-lymphocytes and natural killer T-cells (NKT), that, in turn, stimulate macrophage activation and the release of pro-inflammatory mediators[6,7]. B-cell are also involved in the process by driving T-cell activation and secreting pro-inflammatory cyto/chemokines[8]. Accordingly, T- or B-cell-null mice are less susceptible to fat inflammation and insulin resistance when fed a high fat diet[9,10].

ROLE OF OXIDATIVE STRESS-MEDIATED IMMUNE RESPONSES IN NAFLD EVOLUTION

In spite of the growing importance of adaptive immunity in stimulating adipose tissue inflammation, so far little attention has been paid to the possible involvement of similar mechanisms in NASH. Oxidative stress is a key feature of NAFLD/NASH[11,12] and many data show that lipid peroxidation products arisen from the oxidation of phospholipids can elicit humoral and cellular immune responses by forming immunogenic adducts through the interaction with cellular proteins[13]. In this regard, our experimental data have shown that 40%-60% of both adults and children with NAFLD/NASH have circulating antibodies against lipid peroxidation-derived antigens, namely malonyldialdehyde (MDA) and 4-hydroxynonenal[14,15]. Noteworthy, high titres of these antibodies are also associated with increased hepatic inflammation, and advanced fibrosis in, respectively children[15] and adults[14]. Similar antibodies against lipid peroxidation-derived antigens are detectable in a dietary rat model of NAFLD and reducing lipid peroxidation by supplementation with the antioxidant N-acetylcysteine prevents antibody response and ameliorates hepatic injury[16]. In line with these observations, experiments using mice with NASH induced by feeding a methionine and choline deficient (MCD) diet demonstrate that $CD4^+$ and $CD8^+$ T-lymphocytes are recruited within the liver and their prevalence parallels the worsening of parechymal injury and lobular inflammation[17]. Both lymphocyte subsets express the CD69 activation marker and $CD4^+$ T-cells show an increased interferon-γ (IFN-γ) production indicating that liver infiltrating lymphocytes have an activated phenotype[17]. Concomitantly, we have also observed a stimulation of humoral immunity with an increase of circulating IgG recognizing antigens derived from protein modification by lipid peroxidation derived adducts[17]. Interestingly, the same antigens are also recognized by hepatic T-cells confirming that oxidative stress can represent the source of neo-antigens able to promote adaptive immune responses[17]. The characterization of the epitopes recognized by circulating antibodies associated with human NASH has revealed that they mainly interact with the cyclic methyl-1,4-dihydroxypyridine-3,5-dicarbaldehyde adducts also known as malonyldialdehyde-acetaldehyde (MAA) adducts[14] which originate by the combined interaction of these aldehydes with protein lysine ε-amino groups[18]. MAA adducts are known to be very antigenic[18]. Furthermore, a recent study by Henning *et al*[19] has shown that the development of MCD-induced NASH is associated with an early expansion in hepatic mature myeloid dendritic cells, which acquire the capacity to specifically stimulate $CD4^+$ T-cells. Thus, this combination can likely drive the activation of adaptive immunity during the onset of NASH. It is noteworthy that immune reactions toward lipid peroxidation-derived epitopes, including the MAA adducts, characterize alcoholic liver disease in both humans and experimental animals[20-22]. In heavy drinkers elevated titres of IgG recognizing lipid peroxidation-related antigens are associated with a 5-fold increase in circulating tumor necrosis factor (TNF)-α levels as compared to subjects without these antibodies and with an enhanced probability to progress to more severe liver injury[23]. Moreover, antigens derived from oxidative modification of low density lipoprotein are also among the triggers of adaptive immune mechanisms involved in the evolution of atherosclerosis and anti-MAA antibodies predict coronary artery disease in atherosclerotic patients[24]. This latter analogy might have clinical relevance in the light of the data suggesting that NAFLD/NASH and atherosclerosis share common pathogenetic mechanisms and the presence of NASH increases the risk of atherosclerotic ischemic complications[25].

INTERACTION BETWEEN INNATE AND ADAPTIVE IMMUNITY IN NASH EVOLUTION

More insides in understanding of the possible role of adaptive immunity in sustaining NASH-associated hepatic inflammation have been obtained by immunizing mice with MDA-derived protein adducts, that are among the antigens related to oxidative stress detected in both in human and experimental NASH. By this approach, we have observed that the stimulation of immune responses worsens parenchymal injury and lobular inflammation in mice receiving the MCD diet and that these effects are associated with an enhanced hepatic lymphocyte infiltration[17]. In these animals, $CD4^+$ T-cell depletion with specific antibodies demonstrates that Th-1 polarized $CD4^+$ T-cells are able to stimulate

hepatic macrophages through the expression of CD40 ligand and IFN-γ[17]. On their turn, by releasing TNF-α, interleukin 12 (IL-12), reactive oxygen species and nitric oxide macrophages further contributes in promoting lymphocyte functions, oxidative stress and parenchymal injury. In fact, $CD4^+$ T-cell ablation in MCD-fed MDA-immunized mice significantly improves liver damage and lobular inflammation[17]. Interestingly, IFN-γ deficiency attenuates steatohepatitis and hepatic fibrosis in mice fed with the MCD diet[26], while interference with CD40/CD154 dyad reduces adipose tissue inflammation in obese mice[27]. Altogether, these results indicate that Th-1 $CD4^+$ T cells activation might represent one of the mechanisms by which adaptive immunity can sustain hepatic inflammation during NASH progression. Overall, these findings are relevant for the human disease since both paediatric and adult NASH are characterized by an up-regulation in liver IFN-γ expression and an increase in circulating $CD4^+$ T-cells producing IFN-γ[28,29]. This does not exclude that additional mechanisms might also be involved. For instance, Tank *et al*[30] have recently reported an increase in Th-17 $CD4^+$ T-cells in mice with steatosis induced by feeding a high fat diet. They also have observed an up-regulation of Th-17 related cytokines (IL-17, IL-21, IL-23) in liver biopsies of NASH patients[30]. In our hands, $CD4^+$ T cells activation in MCD-fed MDA-immunized mice does not affect ROR-γt as well as IL-17a hepatic gene expression[17]. Nonetheless, the actual role of $CD4^+$ Th-17 T-cells in the pathogenesis of NASH requires further investigations, as IL-17 up-regulation has been associated to the pathogenesis of liver fibrosis in mice[31].

Although B-cell depletion reduces fat inflammation in obese mice[10], so far the role of humoral immunity in NASH is poorly investigated. Our preliminary results indicate that in MCD-induced steatohepatitis the presence of antibodies recognizing MDA-antigens is associated with IgG deposition within the inflammatory infiltrates suggesting the possibility that these antibodies may contribute to hepatic damage by inducing antibody-dependent cytotoxicity or complement activation. On this latter respect, Rensen *et al*[32] have recently reported extensive complement activation in liver biopsies of NASH patients, which in turn is associated with enhanced hepatocyte death, granulocyte infiltration and higher liver expression of IL-1β, IL-6 and IL-8 mRNAs. Furthermore, in the livers of immunized mice we have observed that the up-regulation in the B-cell markers parallel with that of pro-inflammatory cytokines. Nonetheless, further investigations are needed to better clarify the possible involvement of humoral responses in NASH.

IS THERE A ROLE OF ADAPTIVE IMMUNITY IN NASH PROGRESSION TO FIBROSIS AND HEPATOCELLULAR CARCINOMA?

In recent years, NAFLD/NASH has not only been recognized to be an important cause of hepatic fibrosis/cirrhosis, but has also been associated with the growing prevalence of the hepatocellular-carcinoma (HCC)[33]. In this latter respect, the possible relevance of adaptive immune mechanisms has emerged from a recent report showing that lymphocyte responses promote HCC development in a dietary model of NASH consisting in mice feeding with a choline deficient high fat diet (CD-HFD)[34]. In this study, Wolf *et al*[34] have observed that NASH and hepatic fibrosis parallel with the liver recruitment of activated $CD4^+$, $CD8^+$ T and NKT cells. Moreover, after 12 mo a substantial fraction CD-HCF-fed animals develops HCC, while $\beta 2m^{-/-}$ mice lacking $CD8^+$ T- and NKT cells are protected from both NASH and HCC[34]. These findings are in line with recent data pointing to an involvement of NKT-cells in the evolution of chronic liver disease[35]. In particular, NKT cell expansion characterizes advanced NASH in both humans and rodents, while MCD-fed NKT cells deficient mice show lower hepatic inflammation and fibrosis than wild type littermates[36,37]. On the same line, we have reported that an increased prevalence of hepatic NKT cells is associated with the worsening of steatohepatitis in MDA-immunized mice receiving the MCD diet[17]. A stimulation in the macrophage production of CXCL16, a chemokine specifically involved in NKT cell recruitment along with an elevation in hepatic levels of IL-15 have been proposed to regulate NKT cell pool in NASH[38,39]. In accord with the importance of IL-15 in supporting T- and NKT cell differentiation and survival[40], we have observed that IL-15 is selectively up-regulated in MDA-immunized MCD-fed mice concomitantly with the expansion of NKT cells, while $CD4^+$ T cell ablation significantly lower the intrahepatic IL-15 mRNA level[17]. This suggests that Th-1 responses might promote NKT cell expansion through IL-15 up-regulation. At present, the mechanisms by which NKT cells contribute to liver injury in NASH are still poorly understood. Syn *et al*[41] have proposed that osteopontin (OPN) producing NKT cells can sustain NASH evolution to fibrosis. An over-expression of liver OPN has been described both in humans and rodents suffering from advanced NASH[41-43], whereas OPN-deficient mice are protected from the development of steatohepatitis and fibrosis induced by the MCD diet[42,44]. In our hands, liver OPN content is selectively up-regulated in immunized MCD-fed mice concurrently with NKT cell recruitment and OPN-producing NKT cells are also increased in the livers of these animals[17]. This could explain the stimulation in collagen deposition that is evident in these animals, as OPN can directly promote collagen production by activated hepatic stellate cells[45]. OPN has also been proposed to induce ductular reaction[46] that is associated with an increased risk of fibrosis evolution in NASH patients[47,48].

CONCLUSION

Available evidence indicates that immune responses stimulated by oxidative stress-related antigens can

sustain the progression of experimental NASH through the Th-1 activation of $CD4^+$ T-cells, which, in turn, stimulate macrophage M1 responses and liver $CD8^+$ T- and NKT cell recruitment. These data along with the findings in humans support the possible contribution of adaptive immunity in the processes driving NAFLD evolution. However, further researches are required to better characterize the interaction between innate and adaptive immunity in sustaining hepatic inflammation and to evaluate the possible application to NASH therapy of the large variety of molecules already under study in other conditions characterized by impaired immune regulation or autoimmunity.

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EDITORIAL

Changing common sense: Anti-platelet/coagulation therapy against cirrhosis

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Abstract

Until recently, anti-platelet/coagulation therapy had not been recommended for patients with cirrhosis. Although venous thrombosis is one of the representative complications of cirrhosis and ischemic disorders associated with atherosclerosis are not infrequent in cirrhotic patients, many clinicians have tended to hesitate to introduce anti-platelet/coagulation therapy to their patients. Undoubtedly, this is due to the increased risk of hemorrhagic diathesis in cirrhotic patients. However, accumulating evidence has revealed the benefits of anti-platelet/coagulation therapy for cirrhotic patients. In addition to the safety of the therapy carried out against cardiovascular diseases in cirrhotic patients, some clinical data have indicated its preventive effect on venous thrombosis. Moreover, the efficacy of anti-platelet/coagulation therapy against cirrhosis itself has been demonstrated both clinically and experimentally. The conceptual basis for application of anti-platelet/coagulation therapy against cirrhosis was constructed through two pathologic studies on intrahepatic thrombosis in cirrhotic livers. It may be better to use thrombopoietin-receptor agonists, which have been tested as a treatment for cirrhosis-related thrombocytopenia, in combination with anti-platelet drugs to reduce the risk of venous thrombosis. During the last decade, the *World Journal of Gastroenterology*, a sister journal of *World Journal of Hepatology*, has been one of the main platforms of active discussion of this theme.

Key words: Anti-platelet/coagulation therapy; Cirrhosis; Hemorrhagic diathesis; Thrombosis; Thrombocytopenia

Core tip: Recognition concerning anti-platelet/coagulation therapy for cirrhotic patients has been changing from relative contraindication to recommendable. Administration of this type of drugs is expected to not only prevent cirrhosis-related thrombotic disorders but also slow down the progression of liver disease itself.

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PROLOGUE: CONTRAINDICATION?

Until recently, it was thought that a balance between the necessity and risks of anti-platelet/coagulation therapy for patients with cirrhosis had to be carefully considered. This therapy was believed to be rather a relative contraindication for cirrhotic patients. Even currently, no one can dispute that this therapy increases the risk of gastrointestinal bleeding in patients with advanced decompensated cirrhosis[1,2]. In contrast, venous thrombosis is one of important complications of cirrhosis[1-7]. In addition, cirrhotic patients who suffer from atherosclerotic cardiovascular and cerebrovascular diseases have been increasing in number with the prevalence of metabolic syndrome. As a result, a certain number of cirrhotic patients need anti-platelet/coagulation therapy. Many physicians seem to administer a minimum amount of anti-platelet/coagulation drugs to such cirrhotic patients very carefully but timidly.

The coagulation status of cirrhotic patients is certainly delicate and is placed on a very sensitive balance. Importantly, the balance easily leans towards coagulable as well as hemorrhagic[8-11].

DISCUSSIONS IN THE *WORLD JOURNAL OF GASTROENTEROLOGY* AND THE COAGULATION IN LIVER DISEASE STUDY GROUP

The accumulation of recent evidence through clinical observations and experimental investigations has been upsetting the hitherto common sense about anti-platelet/coagulation therapy for cirrhotic patients. Previous reports in this research field were mostly negative ones, which emphasized the risks and potential side effects of this therapy[12-14]. In 2003, however, a revolutionary research paper by Shi *et al*[15] published in the *World Journal of Gastroenterology* (*WJG*), a sister journal of the *World Journal of Hepatology* (*WJH*), reported the efficacy of heparin administration to cirrhotic patients. Thereafter, papers suggesting the safety and benefits of anti-platelet/coagulation therapy for cirrhotic patients have been published[6-8,16-30].

An international workshop of the Coagulation in Liver Disease Study Group (CLDSG) established by Professor Caldwell at the University of Virginia, has been promoting this movement. Since 2005, every other year this group has held a symposium with heated discussions to form a consensus for the best management of liver-related coagulation disorders[9,20,21]. Their latest conclusion states that anti-platelet/coagulation therapy is applicable as a treatment and preventive tool for cirrhotic patients although sufficient prophylactic means against gastrointestinal bleeding are required.

After the article of Shi *et al*[15], *WJG* has steadily published further articles suggesting the benefits of this therapy[2,5-8,16,17,22,31,32]. Some papers in *WJG* have discussed the efficacy of this therapy not only for thrombotic complications but also for the diseased liver itself[3,6,17]. The journal and its contributors possessed amazing prospective insight; they were approximately 4 years ahead of analogous publications in other journals[18-20].

THE SOURCE OF THE IDEA

Undoubtedly, two papers written by Wanless *et al*[33,34] and published in 1995 are the source of the present idea that anti-platelet/coagulation therapy may be beneficial for cirrhotic patients. They demonstrated both macroscopically and histologically the presence of intrahepatic thrombi in cirrhotic livers, and suggested that the thrombi potentially contribute to further liver damage (*i.e.*, parenchymal extinction) and to the development and progression of portal hypertension. Subsequently, relevant data indicating abnormal intrahepatic platelet aggregation in cirrhotic patients have been published[20,35,36]. These papers disclose a part of the mechanisms of cirrhosis-related thrombocytopenia, and prove the usefulness of anti-platelet/coagulation therapy for cirrhotic patients.

THROMBOPOIETIN RECEPTOR AGONISTS

As the latest subject in this research field, we refer to clinical trials of administration of thrombopoietin receptor agonists (eltrombopag and avatrombopag) to cirrhotic patients with thrombocytopenia[37-40]. The aim of the interventional trials is to expand the indication of antiviral therapies and invasive procedures for cirrhotic patients[39,40]. These agents obviously increase the circulating platelet count through direct stimulation of thrombopoiesis. Similar to splenectomy for cirrhotic patients, the theoretical background seems to be simple and to make sense. However, since the projects lacked enough insight into coagulation abnormalities of cirrhotic patients, many patients developed portal vein thrombosis[40]. A member of the CLDSG immediately pointed out the significant risk of hypercoagulable complications introduced by non-selective administration of thrombopoietin receptor agonists[41]. A similar discussion was presented in the recent issue of *WJG*[42].

Because the etiology of cirrhosis-related thrombocytopenia is quite diverse[32,36,43,44], forcible normalization of the platelet count targeting only one factor may be either ineffective or even risky. Our recent data[44] suggest that at least three major factors, including decreased thrombopoiesis, hypersplenism and excessive platelet aggregation, contribute to the development of

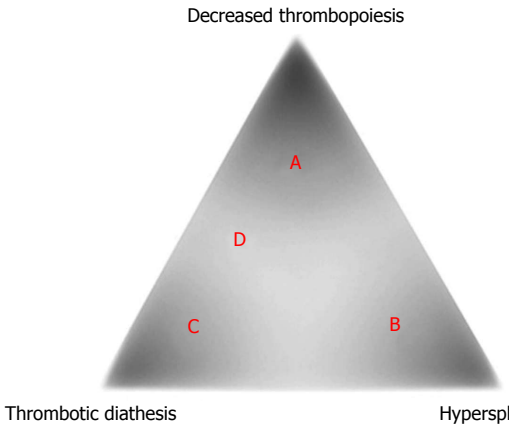

Figure 1 Theoretically ideal therapies against cirrhosis-related thrombocytopenia depending on the etiology. The platelet count in cirrhotics is determined mainly by three major factors, including (A) a rate of thrombopoiesis, (B) the presence/abscence of hypersplenism, and (C) the presence/abscence of thrombotic diathesis, which differently affect each case. Patients with thrombocytopenic conditions A, B, or C are considered to respond well to thrombopoietin receptor agonists, splenectomy, and anti-platelet/coagulation drugs, respectively. In patients with condition D, a combination of thrombopoietin receptor agonists and anti-platelet/coagulation drugs is thought to be necessary.

cirrhosis-related thrombocytopenia (Figure 1). Hence, normalization of the platelet count is thought to require a correct diagnosis of each patient's background conditions leading to thrombocytopenia and strict selection of the appropriate method for each case. In some cases, a combination of multiple methods may lead to a favorable outcome (Figure 1). To construct such a combination therapy, anti-platelet/coagulation therapy is a key element, and needs an approval to the extended application against cirrhosis.

EPILOGUE: TO BE APPROVED AS AN ALTERNATIVE THERAPY

Because platelets are very small blood cells without nuclei and are hardly detected by an ordinary histological examination (Figure 2), their pathological significance in diseases other than vascular disorders has seldom been considered. They play an important role in many inflammatory disorders as a mediator of both inflammatory reactions and fibroproliferative reactions. If hepatologists can correctly understand the robust pathobiological faculties of platelets, a dramatic paradigm shift will be approaching. Anti-platelet/coagulation therapy is not a novel medical tool, and past clinical research suggested the ineffectiveness of this therapy for cirrhosis[45]. However, it should be considered as one of the treatment options for cirrhosis again in this era in which antiviral therapies have sufficiently been developed.

We strongly hope and expect that *WJH* and its sister journal *WJG* will continue to be a discussion platform for and a witness of this changing common sense concerning anti-platelet/coagulation therapy against cirrhosis.

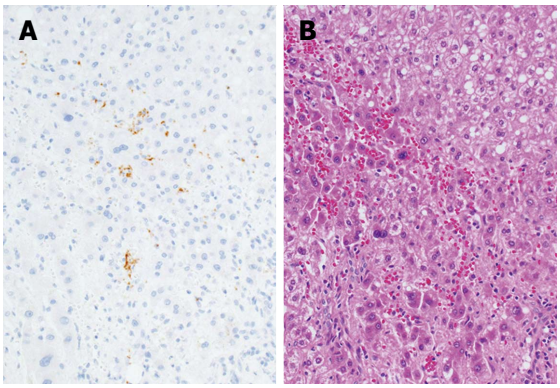

Figure 2 Platelet aggregation in a cirrhotic liver. A: Immunohistochemical findings. Platelets are stained in brown. (Immunoperoxidase for CD41; original magnification, × 400); B: The corresponding histological findings. Platelets cannot be identified in this photomicrograph. (Hematoxylin-eosin; original magnification, × 400).

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EDITORIAL

Pancreaticobiliary reflux as a high-risk factor for biliary malignancy: Clinical features and diagnostic advancements

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Abstract

Pancreaticobiliary junction is composed of complex structure with which biliary duct and pancreatic duct assemble and go out into the ampulla of Vater during duodenum wall surrounding the sphincter of Oddi. Although the sphincter of Oddi functionally prevents the reflux of pancreatic juice, pancreaticobiliary reflux (PBR) occurs when function of the sphincter of Oddi halt. The anatomically abnormal junction is termed pancreaticobiliary maljunction (PBM) and is characterized by pancreatic and bile ducts joining outside of the duodenal wall. PBM is an important anatomical finding because many studies have revealed that biliary malignancies are related due to the carcinogenetic effect of the pancreatic back flow on the biliary mucosa. On the other hand, several studies have been published on the reflux of pancreatic juice into the bile duct without morphological PBM, and the correlation of such cases with biliary diseases, especially biliary malignancies, is drawing considerable attention. Although it has long been possible to diagnose PBM by various imaging modalities, PBR without PBM has remained difficult to assess. Therefore, the pathological features of PBR without PBM have not been yet fully elucidated. Lately, a new method of diagnosing PBR without PBM has appeared, and the features of PBR without PBM should soon be better understood.

Key words: Pancreaticobiliary maljunction; Pancreas juice; Reflux; Flow; Magnetic resonance imaging

Core tip: Pancreaticobiliary reflux (PBR) is an important pathologic state that can cause biliary malignancy. PBR can occur regardless of whether the patient has pancreaticobiliary maljunction (PBM) or not. Although it has long been possible to diagnose PBM by various imaging modalities, PBR without PBM has remained difficult to assess. Therefore, the pathological features of PBR without PBM have not been yet fully elucidated. Lately, a new method of diagnosing PBR without PBM has appeared, and the features of PBR without PBM should soon be better understood.

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INTRODUCTION

The pancreaticobiliary junction is a complex structure composed of the biliary and pancreatic ducts, and is surrounded by the sphincter of Oddi. Although the sphincter of Oddi precludes the reflux of pancreatic juice into the bile duct, pancreaticobiliary reflux (PBR) may occur in case where the functioning of the sphincter of Oddi is impaired[1,2].

An anatomically abnormal pancreaticobiliary junction is termed as a "pancreaticobiliary maljunction" (PBM), and is characterized by the joining of the pancreatic and bile ducts outside the duodenal wall[3,4]. This deformity was first reported in 1916 in case with a choledochal cyst[5]. PBM is an important pathological condition as many studies have indicated that it may be associated with biliary malignancies[6].

Futhermore, several studies have been assessed the reflux of pancreatic juice into the bile duct in the absence of morphological PBM, and the relationship of such cases with biliary diseases, including biliary malignancies, is gaining prominence[2,7-12].

In this editorial, the current knowledge and diagnostic advancements concerning PBR are described.

ETIOLOGY

Anatomy and physiology

The ampulla of Vater is composed of the confluence of the biliary and pancreatic ducts, and the duodenum, and marks the transition from the embryologic foregut to the midgut. The ampulla includes the junction of the common bile duct and the pancreatic duct, the surrounding sphincter of Oddi, the fenestra choledochae, and the duodenal papilla. Although the arrangement of these intersections has some variability, the reported structural relation are common and predictable[13].

The sphincter of Oddi, the regulator of biliary and pancreatic drainage that enable intermittent biliary drainage, originates separately from the duodenal musculature and the periductal mesenchyme, and subseqently becomes incorporated into the duodenal wall. Anatomical studies suggest that the papilla and the duodenal wall contained components specific to the common channel, as well as components specific to the pancreatic and common bile duct that extend outside the duodenal wall.

The common channel is present in 55%-90% of cases[14-16]. The common channel ranges in length from 1 to 12 mm, with an a mean length of approximately 4 mm[14,17,18]. In addition to controlling secretion into the duodenum, the sphincter of Oddi also preclude the mixing of bile and pancreatic juice within the duct system. The long common channels with PBM may cause to become the sphincter mechanism impair and induce the reflux of pancreatic juice, as the pressure of the pancreatic duct is higher than that of the bile duct[2].

However, PBR in patients without PBM is being increasingly recognized[2,7-12], and may develop due to a number of reasons: dysfunction of the sphincter of Oddi, periamupullary diverticula, endoscopic sphincterotomy, and endoscopic papillary balloon dilatation. Of these, most cases of PBR without PBM seem to be caused by dysfunction of the sphincter of Oddi[2].

Etiology of cancer

Although the etiology of biliary cancer in patients with PBR is not yet fully understood, PBR is believed to play an important role in bile duct carcinoma. Once PBR occurs in the pancreaticobiliary junction, the pancreatic and bile juices become mixed and regurgitated mutually, and stagnate in the gallbladder and the bile duct. As a result, activated pancreatic enzymes induce chronic inflammation of the biliary tree and lead to proliferation of the biliary epithelium; moreover this can even cause the expression of carcinogenic substances in the biliary system[19-22]. Therefore, the carcinogenesis process in cases of biliary cancer with PBR is believed to involve the hyperplasia-dysplasia-carcinoma sequence. Several factors that are reportedly related to carcinogenesis in case of biliary cancer have been identified including p53 mutations, microsatellite instability, Bcl-2, and k-Ras point mutations[6,23-28].

CLASSIFICATION

PBR can be divided into two categories on the presence or absence of PBM. PBM can also be divided into two categories on the presence or absence of common bile duct dilatation (Figure 1).

PBM with choledochal cysts

Choledochal cysts are rare congenital biliary tract anomalies formed by biliary tree dilatation. Although the relative frequency of this abnormality in the Western population is 1 in 100000-150000 live births, it is markedly high frequency in Asian countries, particularly Japan, where it may be observed in up to 1 in 1000[29]. The main clinical symptoms include abdominal pain, vomiting, jaundice, and fever. Although the incidence of cholangitis is unclear, cholangitis was observed as a preoperative symptom in 13.2% of patients[6]. Choledochal cysts are commonly divided into several categories on the anatomical features. In 1959, Alonso-lej *et al*[30] devised a classification for choledochal cysts that was modified in 1977 by Todani *et al*[31]. According to Todani's system, choledochal cysts can be divided into five main types. Neverthless, in this system, almost all patients with choledochal cysts fall into three categories (Todani's type Ⅰa, Ⅰc and Ⅳ-A), and are related with PBM.

PBR may contribute to dilatation of the biliary

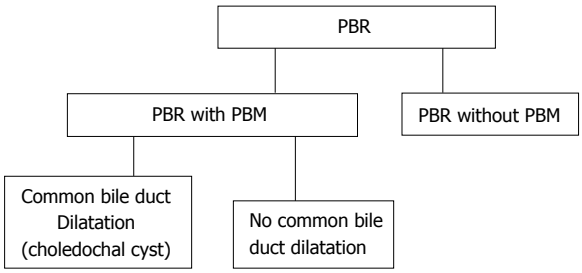

Figure 1 Classification of pancreaticobiliary reflux. PBR: Pancreaticobiliary reflux; PBM: Panceraticobiliary maljunction.

tree[32,33]. Babbit[34] suggested an etiological mechanism for choledochal cyst shaping; it was proposed that enzymes activated in refluxed pancreatic juice induce inflammation, and finaly lead to damage and cystic dilatation of the biliary wall. This theory, however, does not explain the development of all choledochal cysts, as showed by choledochal cysts of newbone infant, which are known to be congenital rather than degenerative.

Biliary tract malignancies are noted in 21.6% of patients with choledochal cysts who are aged > 15 years, whereas biliary malignancies are much rarer in children who present aged < 15 years (0.1%)[6]. Immediate and exact diagnosis of a choledochal cyst, followed by surgical treatment, is crucial consequently.

PBM without choledochal cysts

PBM without a choledochal cyst may result in the reflux of pancreatic juice into the bile duct and may present clinical symptoms similar to those observed in case with PBM with a choledochal cyst. Cholangitis was observed as a preoperative symptom in 8.9% of patients[6]. However, only few patients with PBM without a choledochal cyst have symptoms in childhood; thus, in these patients, PBM tends to be diagnosed at a later stage than in those with a choledochal cyst[6,35]. Pancreatic juice is often refluxed into the biliary duct in PBM patients, and this may be associated with a high frequency of biliary cancer among such patients. While both PBM patients with and those without choledochal cysts are at a risk for biliary malignancies, they differ in the site of malignancy in the biliary tract[6].

In a previous study, biliary cancer was noted in 21.6% of adult patients with choledochal cysts, and in 42.2% of patients with PBM without biliary dilatation. Of all cases of biliary cancer associated with PBM without biliary dilatation, 88.1% were cancers of the gallbladder[6].

PBR without PBM

Lately, several researches on the reflux of pancreatic juice into the bile duct in case without PBM have been published, and the relationship of such a condition with biliary diseases, especially biliary malignancies, is attracting considerable attention[1,2,7-12,36-38]. However, these cases do not present specific clinical symptoms and also could not be acculately detected by using current available imaging modalities based on the morphological changes. Thus, it was difficult to predict and detect PBR without PBM. Generally, PBR in these patients is diagnosed on the basis of elevated pancreatic enzyme levels in bile juice samples, or by using magnetic resonance cholangiopancreatography (MRCP) after secretin injection.

Anderson *et al*[9] demonstrated that elevated amylase levels in bile juice taken from indwelling T-tubes, suggestive of PBR were found in 81% (21 of 26) of patients with biliary disease without PBM. Horaguchi *et al*[8] presented that elevated amylase levels in bile juice after endoscopic retrograde cholangiopancreatography (ERCP) were found in 26% (46 of 178) of patients with a normal junction. Similarly, Sakamoto *et al*[38] detected elevated amylase levels in bile juice juice in 20% (39 of 196) of patients undergoing cholecystectomy without PBM[33]. Kamisawa *et al*[2] focused on the hypothesis that a relatively long common channel without PBM may be related to PBR. They defined a high confluence of the pancreaticobiliary ducts as a common channel length of more than 6 mm, in which communication may be sustained even when the sphincter of Oddi is contracted. They reported that a high confluence of the pancreaticobiliary ducts was found in 1.9% (65 of 3459) of patients who underwent ERCP in their single institute, and that the incidences of gallbladder cancer in patients with a high confluence of the pancreaticobiliary ducts was very high compared to that in controls.

Recently, a multi-center trial in Japan revealed that elevated amylase levels in bile juice after ERCP were found in 5.5% (23 of 420) of patients with a normal junction[36]. This trial showed that the presence of a relative long common channel (not shorter than 5 mm) was the only significant factor for PBR in multivariate analysis, and that the incidence of high amylase levels was significantly higher in patients with gallbladder cancer than in those without gallblader cancer.

DIAGNOSIS

PBM

PBM can be detected with ERCP, ultrasonography (US), endoscopic US (EUS), computed tomography and MRCP. ERCP is the gold standard method for diagnosis of PBM, and pancreatography through the minor duodenal papilla can directly demonstrate pancreatobiliary reflux in PBM patients. When the contrast medium is injected endoscopically through the minor duodenal papilla, it is possible to monitor the reflux of contrast medium into the bile duct through the common channel without outflow into the duodenum[2]. Although ERCP is the diagnostic standard method for PBM, it is somewhat invasive and has a non-negligible risk of morbidity. US can detect choledochal cysts as well as abnormalities of the gallbladder that are possibly associated with PBM. However, because US cannot directly detect PBM, it may

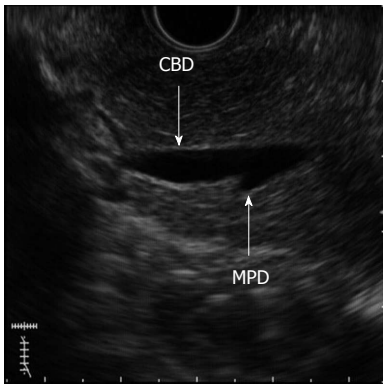

Figure 2 A 56-year-old female with pancreaticobiliary maljunction without choledochal cyst. Endoscopic ultrasound image shows pancreaticobiliary maljunction. CBD: Common bile duct; MPD: Main pancreatic duct.

be suitable for use only as a screening tool. Moreover, EUS has a high spatial resolution and therefore is another modality capable of detecting PBM (Figure 2). However, it is operator dependent and offers a less objective evaluation. In addition, MRCP is a completely non-invasive procedure and causes no adverse reactions due to the contrast medium; however, it may offer a slightly lower spatial resolution than ERCP.

PBR without PBM

Several researchers have described that elevated amylase levels in bile juice samples after ERCP or during the operation (which suggest reflux of pancreatic juice and contrast medium into the pancreatic duct during intraoperative cholangiography) are seen in patients without PBM[1,2,7-12,36]. The normal values of pancreatic enzymes in bile have yet to be fully elucidated. Many researchers use the normal values of pancreatic enzymes in plasma as a reference for detecting PBR without PBM. Some researchers have applyed radioimmunoassays of biliary trypsin in bile samples collected from a T-tube inserted into the common bile duct after surgery[9,39]. Among these methods, the most commonly used method involves the assessmente of bile sample taken directly from the gallbladder at the time of cholecystectomy or ERCP.

However, several researchers have reported that PBR without PBM can be diagnosed by using secretin-stimulating magnetic resonance imaging[10,37]. This method makes it possible to demonstrate pancreatic juice movement on changes in pancreatic or bile duct diameter in consequence of secretin injection. However, this does not visualize pancreatic juice movement directly, and a few controversial reports have therefore questioned the validity of pancreatic juice movement demonstrated by using this method[37,39-41]. Therefore, a robust method is still required.

Advanced diagnosis for detecting PBR without PBM

Recently, a new magnetic resonance-based method (time-SLIP) has been developed for direct visualization of pancreatic juice flow (Figure 3). Initially, this technique allowed the examination of blood flow in vessels to a region of interest[42,43]. This technique could enable the visualization of the flow of bile and pancreatic juices and may allow the evaluation of various pancreatico-biliary diseases based on this information[44,45]. This method revealed new knowledge regarding physiology of bile and pancreatic juices, and was applied for the diagnosis of chronic pancreatitis.

Moreover, this new technique enables the visualization of the reflux of pancreatic juice flow into the bile duct without PBM, although no clinical results have yet been reported (Figure 4). This technique, involving the use of the pancreatic juice as an intrinsic imaging agent, facilitates the examination of pancreatic juice movement similar to more physiolosical situation. In addition, this technique is not time consuming. Accordingly, the method can be easily adopted as a screening tool for PBR. Therefore, this new technique may reveal what proportion of patients without PBM have PBR, and whether reflux is related to biliary carcinogenesis. Further clinical studies are required.

THERAPY

Once PBM is diagnosed, preventive flow-diversion surgery (biloenteric anastomosis and bile duct resection) is excuted for patients with choledochal cysts[4,6]. These operations are often associated with complications such as anastomotic stricture, cholangitis, and secondary liver cirrhosis; therefore, careful follow-up is needed[46].

Nevertheless, any treatment for patients with PBM without biliary dilatation and biliary malignancy is controversial. Preventive cholecystectomy is first choise in many institutions because the majority of biliary malignancies that develop in PBM patients without biliary dilatation are cancers of the gallbladder[4,6,47,48]. On the other hand, excision of the extrahepatic bile duct along with the gallbladder is selected by some surgeons[4,24,49,50]. The treatment for patients with PBR without PBM is not defined because the pathology of PBR without PBM is less well understood. Moreover, strategies for the screening and prevention of PBR without PBM are not yet established.

CONCLUSION

PBR is an important pathologic state that can cause biliary malignancy. PBR can occur regardless of the presence of PBM. Although it has been possible to diagnose PBM by using various imaging modalities, PBR without PBM has remained difficult to assess. Thus, the pathological features of PBR without PBM have not yet been fully elucidated. Recently, a new method for diagnosing PBR without PBM has been introduced, and this would enable a better understanding the feature of

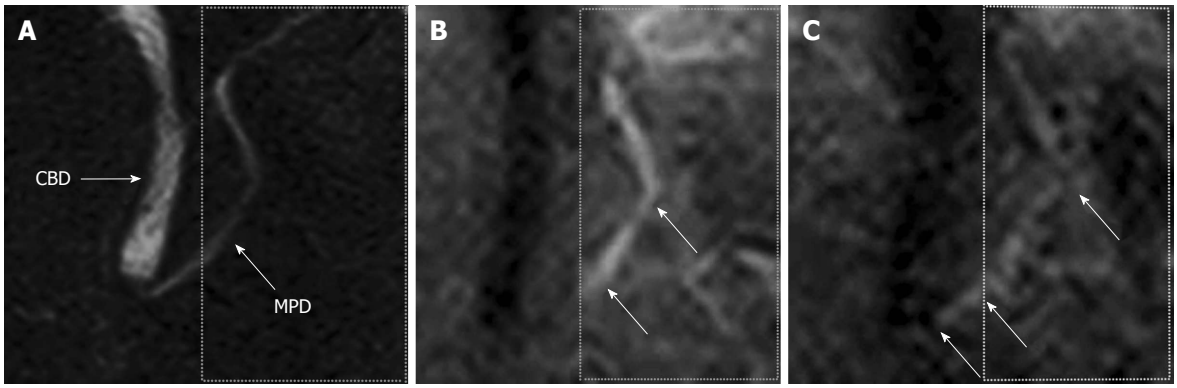

Figure 3 A 47-year-old male with normal volunteer. A: Magnetic resonance cholangiopancreatography image. Labelling pulse of pancreas juice is applying to box surrounded by dotted lines on pancreas juice in body and caudal portion of the 3) main pancreatic duct; B: Time-SLIP image is not showing movement of pancreatic juice; C: Flow of pancreatic juice from body of the pancreas into head of pancreas is noted by high signal intensity (arrows). Reprinted from Sugita *et al*[43] (by permission of Wiley Periodicals, Inc). CBD: Common bile duct; MPD: Main pancreatic duct.

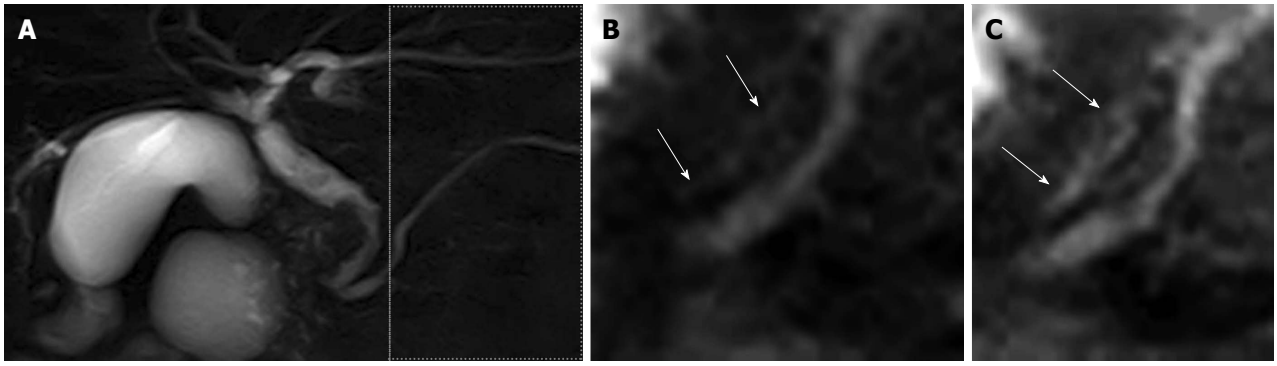

Figure 4 A 75-year-old male with common bile duct carcinoma without pancreaticobiliary maljunction. A: Magnetic resonance cholangiopancreatography image shows the irregular wall throughout the entire common bile duct, with bile duct carcinoma revealed by biopsy. During endoscopic retrograde cholangiopancreatography, amylase level was measured in the bile. The amylase level in the collected bile was 1415 IU/L, higher than the upper limit of serum amylase of 130 IU/L; B and C: Labeled pancreatic juice in the main pancreatic duct refluxing into the common bile duct. Reprinted from Sugita *et al*[43] (by permission of Wiley Periodicals, Inc).

PBR without PBM.

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REVIEW

Nucleos(t)ide analogs in the prevention of hepatitis B virus related hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is among the most common cancer types and causes of cancer related mortality worldwide. Almost 50% of all HCC cases globally are attributable to chronic hepatitis B virus (HBV) infection. The incidence rates of HCC in untreated Asian subjects with HBV infection was estimated to be 0.2% in inactive carriers, 0.6% for those with chronic hepatitis without cirrhosis, and 3.7% for those with compensated cirrhosis. In Western populations, HCC incidences are reported to be 0.02% in inactive carriers, 0.3% in subjects with chronic hepatitis without cirrhosis, and 2.2% in subjects with compensated cirrhosis. Despite effective antiviral treatment options which are able to transform chronic hepatitis into an inactive carrier state, the risk of HCC cannot be fully ruled out to exclude those patients from surveillance. Newer nucleos(t)ide analogues (NAs) as entecavir and tenofovir are very potent in terms of sustained virological suppression which leads to improved liver histology. However, they do not have any influence on the cccDNA or integrated DNA of HBV in the liver. Nonetheless, viral replication is the only modifiable component among the established risk factors for HBV-related HCC with the current treatment options. In this review, it was aimed to summarize cumulative evidence behind the concept of prevention of HBV related HCC by NAs, and to discuss remaining obstacles to eliminate the risk of HCC.

Key words: Hepatitis B virus; Hepatocellular carcinoma; Prevention; Nucleos(t)ide analogues; Risk factors

Core tip: After the introduction of potent nucleos(t)ide analogues with high genetic barrier to resistance, maintaining long-term virological suppression is achievable in almost all patients with chronic hepatitis B. The currently recommended first-line antiviral drugs, entecavir and tenofovir, can significantly reduce hepatocellular carcinoma (HCC) incidence, but the observed risk under efficient therapy is not zero in the long-term. There are established risk factors including age, gender, family history, low platelet levels, presence of cirrhosis or severity of liver disease, which should be incorporated into the clinical decision making to differentiate those patients under risk of developing HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and third most common cause of cancer related mortality worldwide[1]. HCC virtually always develops within a background chronic liver disease. Globally, almost 50% of all HCC cases are attributable to chronic hepatitis B virus (HBV) infection[2]. HCC incidence is highly variable in different geographic locations and the distribution of the disease may be different even among ethnic groups living in the same country[1]. These differences are most probably due to geographical variations in the prevalence of hepatitis viruses. In Sub-Saharan Africa and Asia most cases of HCC develop in the presence of chronic hepatitis B (CHB) infection (up to 60%), however in Western countries only up to 20% of cases can be attributed to HBV infection[2]. Fortunately, after the introduction of universal vaccination programs throughout the world, the incidence of CHB infection was significantly decreased[3]. In the countries that have adopted the HBV vaccination program, a significant decrease in carrier rates as well as complications including HCC development have been experienced[4]. However, HBV infection is still a major health problem in most parts of the world and development of HCC is an important complication of chronic infection, even in inactive carrier patients[5]. In cirrhotic patients, surveillance for HCC increases the possibility of an earlier diagnosis that gives the patient a chance to undergo curative treatments[2]. However, current recommendations for screening of HCC in patients at risk is far from being satisfactory, and prognosis remains poor because treatment options are rather non-curative in advanced stages of the disease[6]. In this context, it is imperative to implement preventive strategies in patients with CHB infection. Despite effective antiviral treatment options which are able to transform chronic hepatitis into an inactive carrier state, the risk of HCC cannot be fully ruled out to exclude those patients from surveillance.

LITERATURE STUDY

In this review, it is sought to summarize the cumulative evidence on the role of antiviral therapy with NAs in the chemoprevention of HCC. Studies on prevention of HBV-related HCC were identified through electronic and manual search using online databases including MEDLINE and Web of Science. The relevant papers and conference proceedings in English language published from January, 2001 to January, 2015 were searched using following keywords: HCC, HBV, prevention, recurrence, curative treatment, resection, nucleos(t)ide analogue (NA), lamivudine, adefovir, telbivudine, entecavir, tenofovir. Selected studies were grouped according to one of the following topics: (1) NAs in chemoprevention of HCC; (2) HCC risk in patients who clear hepatitis B surface antigen (HBsAg); and (3) HCC recurrence risk in patients with HBV-related HCC after curative treatments. Both randomized-controlled and non-randomized studies were considered for inclusion. Uncontrolled studies were excluded unless an estimated HCC risk model was used to assess efficacy of NAs on HCC incidence. Reference lists of all papers, including reviews and meta-analyses, found during electronic search were checked manually to find relevant articles. The papers which were judged to be pertinent to the topic exceeded 100, and the number of included full-length articles were 51.

HBV AND THE RISK OF HCC

The association between HBV infection and development of HCC is well-established and has been demonstrated in several studies. In an early prospective controlled study from Asia, it was shown that the annual incidence of HCC was 0.5% in HBV-infected individuals; and the risk increased with age where annual incidence reached 1% at the age of 70[7]. HBV-infected subjects were found to be 100 times more likely to develop HCC compared to uninfected subjects. The incidence ratio for HCC was even higher as much as 2.5% per year in patients with known cirrhosis. In a recent study by Chen *et al*[5], a lesser but still substantial risk of HCC development was reported in an Asian cohort of patients with inactive HBV infection. In this study, the authors reported an annual incidence rate of 0.06% for HCC development with a relative risk of 4.4 compared to uninfected controls. In Western populations most studies are inadequate to drive conclusions and results are variable. This discrepancy between incidence rates among studies is probably due to the different patient settings (referral or population-based) and definitions for the HBV carrier state. Annual rates as high as 0.47% was reported in a study by Sherman *et al*[8], which can be explained by high prevalence (71%) of Asian background in this North American population. Yet, most studies reported that annual incidence of HBV-related HCC in Europe or North America seems to be less significant[9,10], and it is generally accepted to be around or less than 0.2%[11]. Therefore, it is not clear if surveillance is worthwhile or when is it cost-effective to start screening in Caucasian populations. HBV-infected patients with African ancestry seem to possess a higher risk of developing HCC, particularly at a younger age[12]. A database analysis from the United States reported higher incidence rates among Asians/Pacific Islanders, blacks, Native Americans/Alaska Natives, compared to people with European ancestry[13]. Although HCC may arise in the setting of inactive HBV infection, most patients with HBV who develop HCC have cirrhosis either long-standing or undiagnosed at the time of HCC diagnosis[14,15]. In a review of cohort studies[16], it was summarized that incidence rates of HCC in

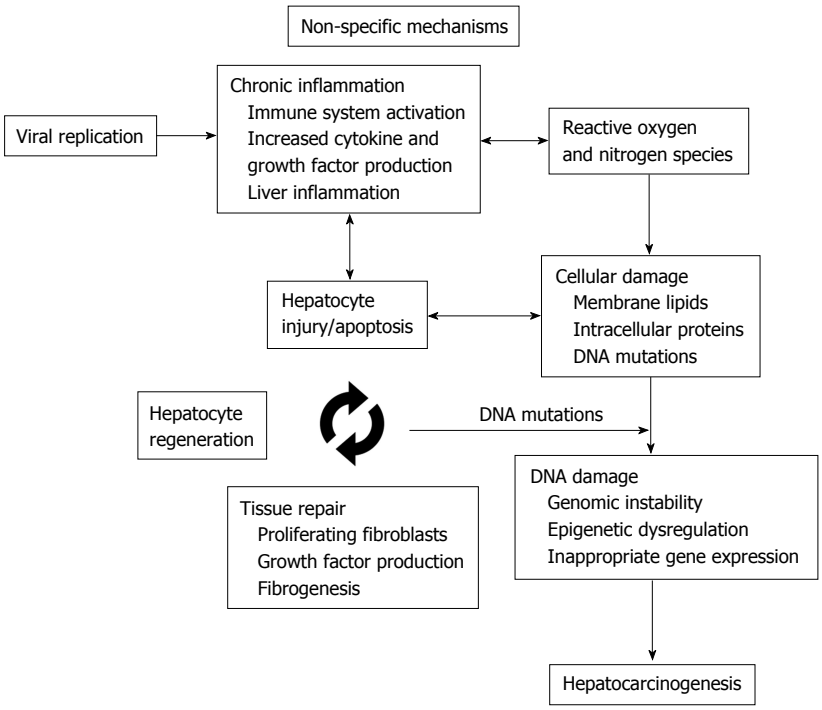

Figure 1 Chronic inflammation in the liver is characterized by sustained damage leading to hepatocyte death/regeneration and tissue repair cycle. Activation of inflammatory signaling with increased cytokine and growth factor production leads to oxidative stress which contributes to cellular damage. Cumulative damage to cellular structures, proteins and chromosomes alters genomic and epigenomic functions which can eventually induce hepatocarcinogenesis.

Asian subjects with HBV infection was estimated to be 0.2% in inactive carriers, 0.6% for those with chronic hepatitis without cirrhosis, and 3.7% for those with compensated cirrhosis. With an attention to inadequacy of studies in Western populations, they calculated an annual incidence rate of 0.02% in inactive carriers, 0.3% in subjects with chronic hepatitis without cirrhosis, and 2.2% in subjects with compensated cirrhosis.

There are a number of factors other than cirrhosis and ethnicity that have been reported to increase HCC risk among HBV carriers. Patient related factors include male gender, older age, high alcohol consumption and family history of HCC, and viral factors include duration of infection, higher viral replication, HBV genotype, and co-infection [hepatitis C, hepatitis D or human immunodeficiency virus (HIV)][14]. Recently, it was also shown that high levels of HBsAg titer (HBsAg $\geqslant$ 1000 IU/mL) is associated with increased risk of HCC in CHB patients with low viral load (HBVDNA < 2000 IU/mL)[17].

MECHANISMS OF HBV-ASSOCIATED HEPATOCARCINOGENESIS

An interaction of complex mechanisms and pathways contribute to the initiation of hepatocarcinogenesis. HBV infection is among the most important risk factors for development of HCC, and can influence carcinogenesis by multiple ways. Chronic inflammation, a driving factor in many types of cancers, is an important pathogenetic mechanism for development of HBV-related HCC. Chronic inflammation in the liver is characterized by sustained hepatic damage, damage-induced apoptosis, hepatocyte death/regeneration cycle, and tissue repair. In addition to continuous cycle of cell death and regeneration, constant activation of inflammatory signaling and increased cytokine production by innate and adaptive immune system contribute to generation of reactive oxygen and nitrogen species which can cause damage to important cellular components including cytoplasmic membrane lipids, intracellular proteins and DNA[18,19]. Genomic instability and alterations in epigenetic regulation of genes can cause inappropriate gene expression and enhanced proliferation of induced cells leading to neoplastic changes in susceptible individuals (Figure 1)[20]. Interestingly, induced cells may have clonal properties which were demonstrated in experimental models that show clonal hepatocyte repopulation is a major risk factor for HCC development[21,22]. In addition to non-specific hepatocarcinogenesis by chronic inflammation, HBV has unique virus-specific mechanisms involving the viral proteins HBx and preS/S, and the insertional mutagenesis with integration of HBV-DNA into the host genome that alters the expression of endogenous genes or induces chromosomal instability, and causes epigenetic changes including alterations in genomic methylation and regulation of microRNA expression (Figure 2)[23].

NAs IN CHEMOPREVENTION OF HCC

The real key for certain prevention of HBV-related HCC is vaccination against the virus for newborns and people at risk. However in the absence of definitive curative treatment, there is a need for accurate risk estimation

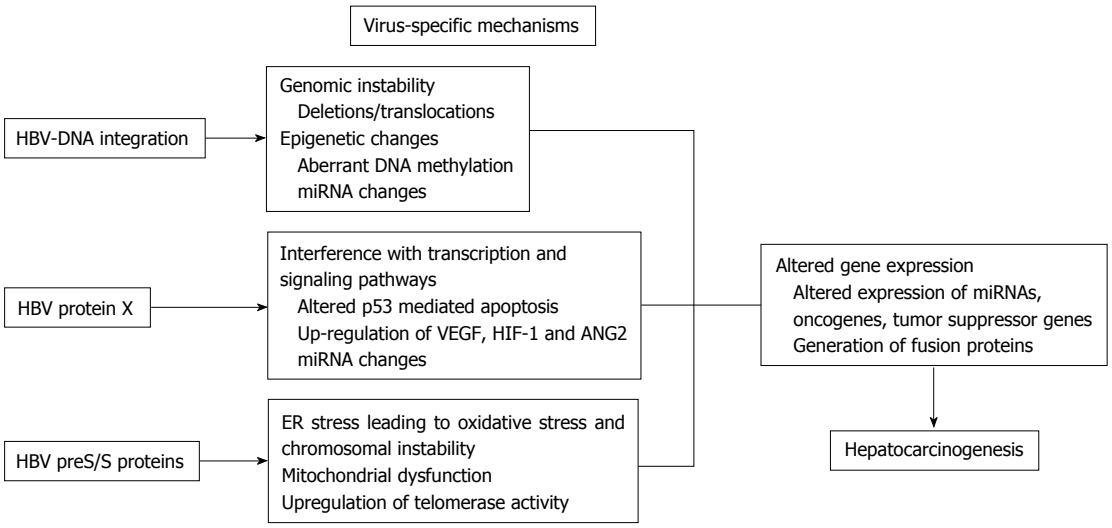

Figure 2 Hepatitis B virus has unique virus-specific mechanisms to induce carcinogenesis in the liver. The integration of hepatitis B virus (HBV)-DNA into the host genome alters DNA expression by gene and/or chromosomal deletions/translocations, and epigenetic changes (aberrant DNA methylation, miRNA changes). Viral protein HBV protein X may cause interference with transcription and signaling pathways leading to altered p53 mediated apoptosis and angiogenesis by up-regulation of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1 (HIF-1) and angiopoietin-2 (ANG2). HBV preS/S proteins cause endoplasmic reticulum stress and mitochondrial dysfunction leading to oxidative stress.

and modification in patients with chronic infection. Among the established risk factors for HBV-related HCC, patient or virus-related factors cannot be modified except viral replication. In a large prospective cohort by REVEAL-HBV study group, the relationship between HBV-DNA titer and HCC risk was demonstrated without any doubt[24,25]. Currently, there are five NAs approved for the treatment of patient with CHB: lamivudine, telbivudine, adefovir, entecavir and tenofovir disoproxil[26]. In the era of antiviral drugs with high barrier to resistance, we can suppress HBV-DNA in almost all patients receiving NAs, but the question remains if treatment prevents the development of HCC in every patient.

Lamivudine is a potent reverse transcriptase inhibitor which was originally developed for the treatment of patients with HIV infection. It is the first oral antiviral drug that was approved for treatment of patients with CHB. Lamivudine monotherapy in patients with CHB is associated with ALT normalization, HBV-DNA suppression, hepatitis B "e" antigen (HBeAg) sero-conversion[27,28], and histological improvement (regression of necroinflammation and fibrosis) in the long-term[29]. The role of NAs to prevent HCC has been thoroughly investigated in multiple studies but the largest number of studies available considered only lamivudine. The studies which investigated effects of NAs in reduction of HCC risk in CHB are summarized in Table 1. In a study by Liaw *et al*[30], which evaluated long-term benefits of lamivudine monotherapy included 651 CHB patients with biopsy-proven advanced fibrosis or cirrhosis in a prospective randomized-placebo controlled setting. This pivotal study was terminated early due to significant beneficial effects seen in the treatment arm. Specifically, HCC development was observed in 3.9% of the patients in the treatment group and 7.4% of those in the placebo group with a hazard ratio of 0.49 (P = 0.047). In the same year, AISF (Italian Association for the Study of Liver Disease) Lamivudine Study Group investigated the effect of lamivudine treatment on the outcome of patients with HBeAg-negative CHB in a multicenter retrospective study[31]. They found that cirrhotic patients with maintained virological response were less likely to develop HCC and disease worsening. But, presence of cirrhosis and virological breakthrough were independently related to mortality and development of HCC. In another randomized-controlled trial by Chan *et al*[32], HBeAg-negative CHB patients were enrolled to lamivudine (100 mg/d) or placebo arms to investigate the efficacy of 2-year lamivudine treatment. Apparently, they did not find a risk reduction for HCC, but the study was not designed to answer this question and sample size was too small to get conclusions. Owing to undeniable beneficial effects of lamivudine therapy in CHB, subsequent randomized-controlled studies cannot be conducted to evaluate the influence of NAs. Thereafter, multiple case-control studies and prospective or retrospective cohort studies using historical controls were published. In a retrospective cohort study in Japan by Matsumoto *et al*[33], which included 377 treated patients (lamivudine 100 mg/d) *vs* 377 matched untreated controls showed a significant risk reduction for HCC in treated cohort (0.4% per year *vs* 2.5% per year, $P < 0.001$). Several other Asian cohort studies from China[34,35], South Korea[36-38] and India[39] also confirmed the protective effect of antiviral therapy with lamivudine against development of HCC. The evidence behind HCC chemoprevention with oral antivirals comes largely from Asian cohort studies; however data obtained in Caucasian populations also exists. In a European cohort study from Greece, Papatheodoridis *et al*[40] reported that lamivudine (with adefovir switch or add-on therapy when required) treatment significantly improved survival

Table 1 Summary of controlled studies investigating the effect of nucleos(t)ide analogues on hepatocellular carcinoma risk among treated and untreated patients with chronic hepatitis B

Ref.	Country/ region	Study type	Treatment	No. of patients (T/C)	Patient cohort	Follow-up (T/C), mean or median (yr)	Treatment outcome
Liaw *et al*[30]	Asia	RCT	Lamivudine (100 mg/d)	436/215	Advanced fibrosis or cirrhosis	2.7/2.7	Reduced HCC risk
Manolakopoulos *et al*[80]	Greece	Case-control	Lamivudine (100 mg/d)	30/30	Decompensated cirrhosis	1.5/1.8	No risk reduction
Matsumoto *et al*[33]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	377/377	Chronic hepatitis B (any stage)	2.7/5.3	Reduced HCC risk
Papatheodoridis *et al*[40]	Greece	Retrospective cohort	Lamivudine (adefovir switch or add-on when required)	201/195	HBeAg-negative chronic hepatitis B	3.8/6.1	Better overall survival. Reduced risk of major events including HCC
Chan *et al*[32]	China	RCT	Lamivudine (100 mg/d)	89/47	HBeAg-negative chronic hepatitis B	2.5/2.5	No risk reduction
Yuen *et al*[34]	China	Prospective cohort	Lamivudine (25-100 mg/d)	142/124	HBeAg-positive chronic hepatitis B	7.5/9.0	Reduced cirrhosis/ HCC risk
Lee *et al*[36]	South Korea	Retrospective cohort	Lamivudine (100 mg/d)	589/589	Chronic hepatitis B (any stage)	2.9/5.3	Reduced HCC risk
Ma *et al*[35]	China	Prospective cohort	Lamivudine (100 mg/d)	41/176	Cirrhosis	3.16/NS	Reduced HCC risk
Das *et al*[39]	India	Case-control	Lamivudine or adefovir	151/102	Decompensated cirrhosis	4.0/3.8	Less HCC rate in treated group
Eun *et al*[37]	South Korea	Retrospective cohort	Lamivudine (100 mg/d)	872/699	Chronic hepatitis B (any stage)	4.7/5.7	Reduced HCC risk in cirrhotic patients with SVS
Kim *et al*[38]	South Korea	Retrospective cohort	Lamivudine and/or adefovir, or entecavir	240/481	Cirrhosis	3.9/4.3	Better overall survival. Reduced HCC risk (borderline significance)
Hosaka *et al*[46]	Japan	Retrospective cohort	Entecavir (0.5 mg/d)	316/316	Chronic hepatitis B (any stage)	3.3/7.6	Reduced HCC risk
Wong *et al*[47]	China	Retrospective cohort	Entecavir (0.5 mg/d)	1446/424	Chronic hepatitis B (any stage)	3.0/9.5	Reduced HCC risk in cirrhotic patients
Kumada *et al*[81]	Japan	Retrospective cohort	Lamivudine ± adefovir, entecavir	117/117	Chronic hepatitis B (any stage)	12.3/11.6	Reduced HCC risk
Sievert *et al*[49]	Reg. trial (abstract)	Prospective cohort	Tenofovir (300 mg/d)	641	Chronic hepatitis B (any stage)	6	Reduced HCC risk compared to estimated risk (REACH-B model)
Su *et al*[48]	Taiwan	Prospective cohort	Entecavir (0.5 mg/d)	1123/503	Cirrhosis (HBVDNA > 2000 IU/mL)	3.6/6.8	
Wu *et al*[44]	Taiwan	Retrospective nationwide cohort	Lamivudine, adefovir or entecavir	21595/21595	Chronic hepatitis B (any stage)	3.5/5.2	Reduced HCC risk
Gordon *et al*[82]	United States	Retrospective cohort	94% received NAs, remaining received IFNs	820/1851	Chronic hepatitis B (any stage)	5.2	Reduced HCC risk
Coffin *et al*[83]	United States	Retrospective cohort	NAs	322	Chronic hepatitis B (any stage)	3.2	Reduced HCC risk compared to estimated risk (REACH-B model)

NAs: Nucleos(t)ide analogues; HCC: Hepatocellular carcinoma; T: Treatment group; C: Control group; RCT: Randomized-controlled trial; HBeAg: Hepatitis B e antigen; SVS: Sustained virological suppression; IFNs: Interferons.

and reduced the risk of major events including HCC compared to interferon non-sustained responders and untreated controls.

Despite these encouraging results of lamivudine monotherapy, the development of resistant strains of HBV have been the main problem since it was introduced into the clinical practice. An important finding of the study by Liaw *et al*[30] was that clinical deterioration defined as $\geqslant$ 2 increase in Child-Pugh score was significantly more frequent in patients who develop YMDD mutation under lamivudine treatment. In a large cohort study from South Korea, lamivudine treatment reduced the incidence of HCC both in patients with CHB and cirrhosis, yet the risk reduction was significant only for compensated cirrhotic patients and when the viral suppression was sustained[37]. Patients with virological breakthrough or suboptimal response during lamivudine therapy were shown to have an increased risk for HCC which was comparable to untreated controls. Kurokawa *et al*[41] evaluated 283 patients with CHB treated with lamivudine 100 mg/d in an uncontrolled cohort study. They found that maintained virological response, presence of cirrhosis, and age are

independent risk factors for development of HCC in patients under lamivudine therapy.

In the era of NAs with high genetic barrier against resistance, entecavir and tenofovir are the only oral antiviral drugs recommended by major society treatment guidelines for CHB[26,42,43]. However, the evidence behind these novel oral antivirals regarding HCC risk reduction is scarce. In the largest retrospective nationwide CHB cohort from Taiwan[44], the investigators included 21595 matched patients in the treatment and control groups. Most of the patients were treated by lamivudine, yet 5748 patients received entecavir 0.5 mg/d. The treated cohort had a significantly lower 7-year incidence of HCC (7.32%; 95%CI: 6.77%-7.87%) than controls (22.7%; 95%CI: 22.1%-23.3%; $P < 0.001$). After adjusting for confounding factors, NA treatment was associated with a reduced risk of HCC, with an adjusted hazard ratio of 0.37 (95%CI: 0.34-0.39; $P < 0.001$). However, the authors did not report if there were any differences between lamivudine and entecavir treated patients regarding HCC incidence. In a randomized trial of 191 patients with decompensated cirrhosis, entecavir-treated patients tended to have a lower incidence of HCC than those treated with adefovir, but the risk reduction was not statistically significant (HR = 0.74, 95%CI: 0.46-1.18, $P = 0.20$)[45]. A recent study from Japan that compared the incidence of HCC in entecavir-treated patients and a matched historical cohort of untreated patients (316 *vs* 316 patients), the investigators found a significantly reduced risk of HCC in the treated group (5 years incidence rates were 3.7% and 13.7% for the treatment and control groups, respectively; $P < 0.001$)[46]. They also compared treatment effect between matched entecavir and lamivudine-treated patients without rescue therapy. It was reported that when the control group was taken as reference HCC risk reduction was more profound in entecavir-treated cirrhotic patients than it is for lamivudine-treated cirrhotic patients ($P < 0.001$ *vs* $P = 0.019$). Of note, this effect was seen in cirrhotic patients but not in non-cirrhotics. In a study from China by Wong *et al*[47], the cumulative probability of HCC between entecavir and untreated historical controls were comparable ($P = 0.82$). However, entecavir-treated patients with radiological cirrhosis had a significantly lower 5-year cumulative probability of HCC (13.8% *vs* 26.4%, $P = 0.036$) compared to the untreated patients with cirrhosis. This effect was more profound in cirrhotic patients with maintained virological suppression. On the other hand, entecavir-treated patients with cirrhosis who failed to achieve undetectable HBV-DNA had a comparable risk of HCC with the untreated patients. In a recent multicenter study from Taiwan[48], 1123 patients with HBV-related cirrhosis treated with entecavir were compared to 503 historical controls with HBV-related cirrhosis. All patients had baseline serum HBV-DNA level > 2000 IU/mL. Although treated patients were significantly older and had more advanced liver disease compared to historical controls, entecavir treatment was shown to be associated with an adjusted hazard ratio of 0.40 (95%CI: 0.27-0.60) in cirrhotic patients. After adjusting for age, the multivariate analysis showed that male gender, no treatment, lower albumin level and lower platelet count were independent risk factors associated with HCC development.

There are very few data regarding treatment effect of tenofovir on HCC incidence. One of them comes from post-hoc analysis of the registration trial of tenofovir, which was reported as an abstract. In this report by Sievert *et al*[49], investigators included 641 patients with CHB receiving open-label tenofovir therapy for 6 years and compared HCC incidence with the estimated risk calculated by REACH-B model[50]. They reported that 14 tenofovir-treated patients (6 of them were cirrhotic) developed HCC during the follow-up and the incidence of HCC decreased with a standardized incidence ratio of 0.45 (95%CI: 0.23-0.91) compared to the estimated risk. Despite the low number of HCC cases in this study, they concluded by emphasizing continued surveillance for CHB patients receiving long term oral antiviral treatment.

There are several meta-analyses or systematic reviews that confirmed the beneficial effects of NAs in the prevention of HCC in CHB. In a systematic review by Papatheodoridis *et al*[51], 21 studies were reviewed and 3 of them which were of high quality and included untreated controls. In the pooled analysis of 3 studies, HCC was detected significantly less frequently in treated than in untreated patients (2.8% *vs* 6.4%, respectively, $P = 0.003$). Interestingly, they found that incidence of HCC was significantly higher in untreated patients even when compared to treated patients with virological breakthroughs or no response. Similarly, other meta-analyses confirmed these results regarding the influence of NAs on HCC risk (Table 2)[52-54].

Although it is evident to say that antiviral therapy decrease HBV-related HCC incidence when compared with the natural course of the disease, there are sufficient data showing that antiviral therapy does not eliminate the HCC risk completely, even in non-cirrhotic patients with sustained virological suppression. Papatheodoridis *et al*[55] included 818 patients with HBeAg-negative CHB treated with NAs in a retrospective study investigating HCC incidence. All patients were treated with NAs starting with lamivudine monotherapy, and during a median follow-up of 4.7 years 49 patients (6%) eventually developed HCC. The study demonstrated a trend for lower cumulative HCC incidence in CHB patients with virological on-therapy remission ($P = 0.076$), which was defined as maintained undetectable HBV-DNA (< 200 IU/mL). However, virological remission did not significantly influence the incidence of HCC in patients with cirrhosis (0.327). Moreover, multivariate analysis revealed that age, gender and cirrhosis were independently associated with HCC risk regardless of virological remission. In a recent study by Arends *et al*[56], 14 of 744 patients (42% Caucasian ethnicity) with CHB developed HCC during a median follow-up of 167 wk. Nine (64%) patients among them had

Table 2 Systematic review and meta-analyses investigating hepatocellular carcinoma risk reduction in patients receiving nucleos(t)ide analogues *vs* untreated controls

Ref.	No. of studies	No. of patients (T/C)	OR (95%CI)	*P*
Papatheodoridis *et al*[51]	3	1313 (779/534)	0.43 (0.25-0.74)	0.002
Zhang *et al*[52]	6	3644 (2035/1609)	0.26 (0.15-0.47)	< 0.00001
Singal *et al*[53]	6	6877 (3306/3571)	0.48 (0.38-0.61)	< 0.00001
Sung *et al*[54]	5	2289 (1267/1022)	0.22 (0.10-0.50)	0.0003

T: Treatment group; C: Control group.

cirrhosis at baseline, and 12 patients developed HCC even after achieving virological response (HBV-DNA < 80 IU/mL). The 5-year cumulative incidence rate of HCC was reported to be low for non-cirrhotic patients, yet it was significantly higher for cirrhotic patients (2.1% *vs* 10.9%, respectively, $P < 0.001$).

Several studies investigated independent risk factors associated with HCC development in patients receiving NAs. The factors commonly attributed to HCC risk in patients receiving NAs were age, male gender, duration of disease, presence of cirrhosis and no virological response[41,51,55,57-59]. Recently in a large European retrospective multicenter cohort of 1666 patients with CHB (all Caucasian) treated with entecavir or tenofovir, 71 patients (4.3%) developed HCC within a median follow-up duration of 39 mo (range, 8-140 mo)[60]. The importance of this study is that there was little information available regarding the risk factors of HCC in Caucasian patients treated with novel antiviral drugs. The authors reported an annual incidence rate of 1.37 (95%CI: 1.09-1.73) per 100 patient. The cumulative probability of developing HCC reached 8.7% after 5 years of antiviral therapy. In multivariate Cox regression analysis they found age, male gender, low platelet levels (< 100000/mm^3), and liver disease severity were independently associated with subsequent development of HCC. Of note, they did not find a significant association between virological remission under treatment and risk of HCC development, which was contrary to previous studies. However, this result is probably related to the high virological remission rate (92%) under antiviral treatment. A recent study from Turkey; which investigated the risk of HCC development in 641 CHB patients on-therapy; showed that cirrhosis, NAs with low-genetic barrier against resistance, and development of resistance or virological breakthrough were independent predictors of HCC development[61].

HCC RISK IN PATIENTS WHO CLEAR HBSAG

Whether it is spontaneous or therapy-induced, HBsAg clearance with or without anti-HBs seroconversion is considered highly beneficial. Although HBsAg loss or seroconversion has been the ultimate goal in the treatment of CHB, it is rarely achievable with the available NA options which have no influence on intra-hepatic cccDNA of HBV. Nonetheless, it is still the holy grail of a treatment course which can be achieved by only a small group of patients, even in the long-term. However, until recently data was insufficient to determine if it is reasonable to exclude those patients who achieve HBsAg loss or seroconversion from routine clinical follow-up. Although there is evidence showing that disease progression may still occur after HBsAg loss[62], the common practice have been continuing follow-up and HCC screening only in those with cirrhosis. This seems to be an evidence-based approach, because cirrhosis is the major risk factor for HCC and should be considered a premalignant condition even after HBsAg loss or seroconversion[63]. In a recent study by Simonetti *et al*[64], 1271 Alaskan native patients with CHB were included in a prospective population-based cohort study, and 158 patients achieved HBsAg loss during a mean follow-up duration of 19.6 years. The authors reported that 6 patients (2 of them were cirrhotic) developed HCC during a mean follow-up of 7.3 years after HBsAg clearance. Although the HCC incidence after HBsAg clearance was reported to be significantly lower than the rate in those who remained seropositive, the risk was still high enough to justify continuum of periodic follow-up visits with HCC surveillance.

HCC RECURRENCE RISK IN PATIENTS WITH HBV-RELATED HCC AFTER CURATIVE TREATMENTS

Despite advances in surgical techniques, survival after curative resection for HBV-related HCC remains dissatisfactory with recurrence rates of more than 50%[65]. There is no proven adjuvant chemotherapeutic regimen that can reduce recurrence risk or improve patient survival after curative resection. However, the above mentioned body of data provides solid evidence that antiviral therapy in patients with CHB or cirrhosis reduces the risk of HCC development, leading to the notion that antiviral therapy might prevent recurrence and improve survival after curative therapy. There is convincing evidence that persistent high viremia is associated with increased risk of HCC recurrence in CHB patients who underwent resection for HBV-related HCC[66,67]. Therefore, the aim has been focused on suppression of persistent HBV replication, which

Table 3 Summary of controlled studies investigating the efficacy of nucleos(t)ide analogues in prevention of hepatocellular carcinoma recurrence after curative treatments

Ref.	Country	Study type	Antiviral treatment	No. of patients (T/C)	HCC treatment	Follow-up (T/C), mean/median (yr)	Treatment outcome
Piao *et al*[69]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	30/40	Resection or ablation/TACE	Not specified	No risk reduction for HCC recurrence Better overall survival
Shuqun *et al*[70]	China	Retrospective cohort	Lamivudine (100 mg/d) + thymosin α1	17/16	Resection	1-3	Reduced HCC recurrence (NS)
Kuzuya *et al*[71]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	16/33	Resection or RFA	3.2/2.7	No risk reduction
Kubo *et al*[72]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	14/10	Resection	2.1	Higher tumor-free survival
Yoshida *et al*[84]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	33/71	RFA	2.8/3.9	No risk reduction
Hung *et al*[85]	China	Retrospective cohort	Lamivudine (100 mg/d)	10/62	Resection	1.6	Reduced HCC recurrence
Koda *et al*[86]	Japan	Retrospective cohort	Lamivudine or entecavir	30/20	Resection or ablation/TAE	2.4/3.0	No risk reduction for HCC recurrence Better overall survival
Chuma *et al*[87]	Japan	Retrospective cohort	Lamivudine (100 mg/d)	39/64	Resection or RFA	2.9/4.4	Reduced HCC recurrence
Li *et al*[74]	China	Prospective cohort	Lamivudine ± adefovir	43/36	Resection	1.0	No risk reduction
Chan *et al*[73]	China	Retrospective cohort	Lamivudine or entecavir	42/94	Resection	Not specified	Reduced HCC recurrence
Urata *et al*[88]	Japan	Retrospective cohort	Not specified	46/24[2]	Resection	3.1	Tumor-free survival is better *vs* patients with high viral load
Yang *et al*[89]	China	Prospective cohort	Lamivudine, adefovir or entecavir	142/188	Resection	4.0	Reduced HCC recurrence
Wu *et al*[75]	Taiwan	Retrospective nationwide cohort	Nucleoside analogue(s)	518/4051	Resection	2.6/2.2	Reduced HCC recurrence
Huang *et al*[90]	China	Prospective cohort	Lamivudine, adefovir or entecavir	865/175	Resection (HBVDNA > 2000 IU/mL)	3.5	Better disease-free survival (borderline significance) Better overall survival
Huang *et al*[68]	China	Retrospective cohort	Lamivudine, adefovir or entecavir	150/1459	Resection (HBVDNA < 2000 IU/mL)	2.9-3.3	Better disease-free survival
Ke *et al*[91]	China	Retrospective cohort	Lamivudine (100 mg/d)	141/337	Resection	2.0/1.9	No risk reduction for HCC recurrence Better overall survival
Su *et al*[92]	Taiwan	Retrospective cohort	Lamivudine, entecavir or pegylated interferon	62/271	Resection	3.8	Reduced HCC recurrence Better overall survival
Yin *et al*[76]	China	2 cohorts (RCT and NRC)	Lamivudine[1] (100 mg/d)	RCT: 81/82; NRC: 215/402	Resection	RCT: 3.3; NRC: 1.98	Reduced HCC recurrence and better overall survival in both cohorts
Yeh *et al*[93]	Taiwan	Retrospective cohort	Entecavir, lamivudine, telbivudine, or combination	490/3369	Resection, RFA, PEI	3.3/3.3	No benefits for HCC progression or overall survival
Zhang *et al*[94]	China	Retrospective cohort	Entecavir (0.5 mg/d)	40/47	Resection	2.6	Reduced recurrence if HCC ≤ 3 cm
Hann *et al*[95]	United States	Retrospective cohort	NAs	16/9	Resection, RFA, PEI, TACE	5.0	Reduced HCC recurrence Better overall survival
Nishikawa *et al*[96]	Japan	Retrospective cohort	Lamivudine, adefovir or entecavir	99/32	Resection, RFA, PEI	3.5/4.0	No risk reduction for HCC recurrence Better overall survival
Huang *et al*[77]	China	RCT	Adefovir (10 mg/d) Switch to entecavir (18 patients)	100/100	Resection	5.0	Reduced HCC recurrence Better overall survival
Chong *et al*[97]	China	Retrospective-prospective cohort	Nucleoside analog(s)	254/150	Resection	3.3/3.6	No risk reduction for HCC recurrence Better overall survival

[1]Adefovir ± lamivudine or entecavir for drug resistance; [2]13 patients with high viral load, 11 patients with low viral load. HCC: Hepatocellular carcinoma; T: Treatment group; C: Control group; NS: Not significant; TACE: Transarterial chemoembolization; TAE: Transarterial embolization; RFA: Radiofrequency ablation; PEI: Percutaneous ethanol injection; NAs: Nucleos(t)ide analogues; RCT: Randomized-controlled trial; NRC: Non-randomized cohort.

Table 4 Summary of meta-analyses which investigated preventive effect of nucleos(t)ide analogues on hepatocellular carcinoma recurrence in patients who underwent curative treatments

Ref.	No. of studies	No. of patients (T/C)	OR (95%CI)	*P*
Miao *et al*[98]				
1 yr recurrence	2	119 (46/73)	0.59 (0.24-1.43)	0.24
2 yr recurrence	2	119 (46/73)	0.82 (0.34-1.74)	0.60
Wong *et al*[65]	9	555 (204/347)	0.59 (0.35-0.97)	0.04
Sun *et al*[78]	13	6350 (1227/5123)	0.66 (0.54-0.80)	< 0.0001
Zhou *et al*[79]	8	6127 (NS)	0.69 (0.59-0.80)	< 0.00001

NS: Not significant; T: Treatment group; C: Control group.

occurs in almost all patients after liver resection and can severely reduce liver function and survival[68]. Initial studies with small sample sizes did not find any significant effect of NAs to prevent HCC recurrence[69-71]. In the first study that demonstrated a beneficial effect, Kubo *et al*[72] found a lower 5-year disease-free survival rate after surgery in the lamivudine-treated group than control group. Subsequent studies provided conflicting results most probably due to the insufficient number of patients included, treatment heterogeneity (resection, local ablation, chemoembolization), and short duration of follow-up after resection (Table 3). For example, Chan *et al*[73] reported a significantly higher 5-year tumor-free survival rates in the treatment group (42 patients, lamivudine 100 mg/d or entecavir 0.5 mg/d) than in the control group (94 patients) (51.4% *vs* 33.8%, respectively, $P = 0.05$). In contrast, Li *et al*[74] demonstrated a higher but insignificant 1-year tumor free survival after curative hepatectomy in treated (Lamivudine ± adefovir) *vs* control groups (23.3% *vs* 8.3%, respectively, $P = 0.072$). Yet, the duration of follow-up after resection was too short to get conclusions. A larger study with a longer duration of follow-up in a retrospective nationwide cohort by Wu *et al*[75] demonstrated a significantly reduced HCC recurrence rate in patients receiving NAs after liver resection. Two recently published randomized-controlled trials and several meta-analyses (Table 4) also confirmed these results which were obtained from retrospective cohort studies[65,76-79].

CONCLUSION

In terms of HBV and hepatocarcinogenesis, it is important to emphasize that unlike other chronic liver diseases, HCC is not always seen on a background of advanced fibrosis or cirrhosis in CHB patients. Many studies confirmed the concept of suppression of HBV replication for chemoprevention of HCC in either primary or secondary prevention settings. Many of those studies are not perfect, but cumulative evidence shows an undeniable beneficial effect of NAs. Although the effect of modern NAs cannot be assessed in prospective-controlled trials which include placebo-treated or untreated patients in the control groups, there is no doubt that entecavir or tenofovir can provide greater and long-term virological suppression leading to reduced HCC incidence and better patient survival. Regardless of the antiviral treatment, several risk factors including but not limited to old age, male gender, longer duration of the disease, inadequate virological suppression and presence of cirrhosis are clearly associated with the ongoing risk of HCC development. Fortunately, inadequate virological suppression is not an issue with the newer antivirals, if patients are adherent with their medications. Recent evidence also shows that treatment with NAs cannot completely wipe out the risk of HCC, even in patients without risk factors or patients who clear HBsAg. Therefore, future studies should focus on differentiating patients who remain under risk despite effective antiviral therapy and providing cost-effective surveillance strategies in those patients under risk of HCC.

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MINIREVIEWS

Surgical treatment of intra hepatic recurrence of hepatocellular carcinoma

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Abstract

Recurrence after hepatocellular carcinoma (HCC) is frequent. Currently, there are no recommendations on therapeutic strategy after recurrence of HCC. Whereas the 5 year-recurrence rate after resection of HCC is 100%, this drops to 15% after primary liver transplantation. Repeat hepatectomy and salvage liver transplantation (SLT) could be performed in selected patients to treat recurrent HCC and enable prolonged overall survival after treatment of recurrence. Other therapies such as local ablation, chemoembolization or sorafenib could be proposed to those patients unable to benefit from resection or SLT. A clear definition of the place of SLT and "prophylactic" liver transplantation is required. Indeed, identifying risks factors for recurrence at time of primary liver resection of HCC may help to avoid recurrence beyond Milan criteria and non-resectable situations. In this review, we summarize the recent data available in the literature on the feasibility and outcomes of repeat hepatectomy and SLT as treatment for recurrent HCC.

Key words: Hepatocellular carcinoma; Liver resection; Survival; Intrahepatic recurrence; Liver transplantation

Core tip: Recurrence after hepatocellular carcinoma (HCC) is frequent. Repeat hepatectomy and salvage liver transplantation (SLT) could be performed in selected patients to treat recurrent HCC and enable prolonged overall survival after treatment of recurrence. A clear definition of the place of SLT and "prophylactic" liver transplantation is required. Identifying risks factors for recurrence at time of primary liver resection of HCC may help to avoid recurrence beyond Milan criteria and non-resectable situations. In this review, we summarize the recent data available in the literature on the feasibility and outcomes of repeat hepatectomy and SLT as treatment for recurrent HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumor. Worldwide, it represents the sixth most common cancer in terms of incidence and is the second cause of cancer-related death[1].

The European Association for the Study of the Liver-European Organisation for Research and Treatment of Cancer group has defined therapeutic strategies for management of primary HCC with the Barcelona-Clinic Liver Cancer (BCLC) algorithm[2]. Currently, there are no published guidelines for treatment of recurrent HCC. Furthermore, there are no randomized prospective studies available in the literature, to determine treatment of choice. In absence of guidelines, some authors have used the BCLC algorithm for cases of recurrent HCC. As for primary tumors, different treatments could be performed in cases of recurrent HCC: second or more resection, salvage liver transplantation (SLT), local ablation, chemoembolization or antiangiogenic therapy. In this short review, we highlight potential surgical treatments for recurrent HCC, including second resection or liver transplantation.

RECURRENT HCC

Tumor recurrence is common after primary resection of HCC. After HCC resection, the 5-year survival rate is about 70%. However, the rate of recurrence remains high, with a 5-year cumulative rate of up to 100%[3]. After liver transplantation, the 5-year recurrence rate is estimated at between 5% and 15% in the literature[4-6]. In addition, no adjuvant treatment is recommended to prevent recurrence.

Recurrence is confined to the liver in 80% to 95% of cases. In about 50% of cases, recurrence is multifocal. In 15% of cases, extra hepatic recurrence is associated[7,8]. Two types of recurrences may be distinguished: early recurrence and late recurrence[9]. The literature is unclear regarding cut-off time with some authors considering cut-off at 12 mo and others 2 years. Usually, early recurrence is considered to occur up to 2 years after primary HCC and late recurrence more than 2 years after primary resection. Early recurrence and late recurrence are considered to represent different mechanisms of recurrence. Early recurrence is considered as metastatic occurrence and late recurrence as multicentric occurrence of HCC[9]. These two types of recurrences seem to have different outcomes suggesting different treatments. Risk factors for both early and late recurrence have been reported in the literature. Microvascular invasion, satellite nodule, poor differentiation, nonanatomic resection are risk factors for early recurrence. Late recurrence shares the same risk factors as primary HCC[9,10]. According to the literature, recurrence within Milan criteria after primary resection of HCC ranges between 60% to 80%[11,12].

Surgical treatment of recurrence

Two surgical treatments may be considered for recurrent HCC: re-resection and liver transplantation. Because of the lack of available grafts, few patients are able to benefit from treatment by transplantation.

Re-resection is considered as the treatment of choice for patients with intra hepatic recurrence and well-preserved liver function, while liver transplantation is mostly performed in patients with poor liver function. However, some authors recommend other therapeutic strategies.

REPEAT HEPATECTOMY

Only about 20% of patients with recurrent HCC are candidates for surgical treatment. Localization of the tumor, number of tumors, and liver function determine choice of treatment for recurrent HCC. In cases of multinodular liver recurrence, repeat resection should not be recommended.

Repeat liver resection is reserved for patients with good liver function and sufficient estimated remnant liver volume (RLV) after re-resection. If the volume of the future liver remnant is estimated to be inadequate, portal embolization may increase the volume of the future liver remnant. If the change in volume is insufficient, repeat hepatectomy is absolutely contraindicated and another treatment should be considered. In cirrhotic liver, RLV should be more than 40% of total liver volume. In a retrospective study, Lin *et al*[13] suggested a cut-off of the ratio of RLV-body weight of 1.4% to avoid postoperative liver failure.

In 1986, Nagasue *et al*[14] was the first to report a series of repeat hepatic resections for recurrent HCC without mortality. Subsequent progress in hepatobiliary surgery and methods for evaluating liver function led to publication of several series of patients with repeat resection for recurrent HCC.

In 2013 Chan *et al*[15] published, a systematic review of the outcomes of repeat hepatectomy. This review included series between January 2000 and November 2012. Twenty-two series were identified, with no randomized trial. This review confirmed the feasibility of repeat hepatectomy in patients with cirrhotic liver. The median morbidity range of this review was between 0% and 6%, confirming the safety of repeat hepatectomy. Ascites was the most frequent morbidity with a median range of between 0% and 32%.

Since this publication, other series on repeat hepatectomy have been published, notably two major series on second or more hepatectomies to treat repeat hepatectomy. Mise *et al*[16] report a study on third or more hepatectomies for recurrent HCC. The results of three hepatectomy groups were compared: first hepatectomy, second hepatectomy, and third hepatectomy or more. In this study, no 90-d mortality was reported for either second hepatectomy or third hepatectomy and more. No significant difference in morbidity rate was found between the different hepatectomy groups. The morbidity rate of patients after second hepatectomy was 18% in 289 patients and the morbidity rate after third hepatectomy or more was

Table 1 Major studies of outcomes of repeat hepatectomy for recurrent hepatocellular carcinoma from 2010

Ref.	Type of study	Patients (*n*)	Treatment	5 yr overall survival	Prognostic factors for time to recurrence
Zhou *et al*[26]	Systematic review	1149	Re resection	48.5% (25%-87%)	Female gender Younger age Tumor grade Microvascular invasion Recurrent tumor > 3 cm Albumin < 35 g/L
Huang *et al*[23]	Retrospective study	82	Re resection	22.4%	Microvascular invasion
Chan *et al*[15]	Systematic review	1125	Repeat hepatectomy	52% (22%-83%)	Blood transfusion Macro/microvascular invasion Tumor number Tumor size Liver status
Yamashita *et al*[17]	Retrospective study	163	Second hepatectomy	60%	
		46	Third or more hepatectomy	43%	
Tabrizian *et al*[27]	Retrospective study	356	Re resection: 19%	47%	Type of treatment
			Transplant listing: 16%	51%	Tumor number
			Local ablation: 17%	25%	Tumor size
			Embolization: 23%	9%	Alphafoeto protein rate
			Other: 12%	0%	
			None: 7%	0%	
Mise *et al*[16]	Retrospective study	289	Second hepatectomy	60.5%	Satellite nodules
		110	Third or more hepatectomy	68.5%	

23% in 110 patients[16].

In a retrospective study, Yamashita *et al*[17] compared the results of repeat hepatectomy for recurrent HCC. Second hepatectomy was performed in 163 patients and third hepatectomy or more in 46 patients. The mortality rate after second hepatectomy was 1.2%, compared to 0% mortality rate after third or more hepatectomy. No significant difference was found between the three groups in terms of morbidity. The morbidity rate was 26% for second and 30% for third hepatectomy[17].

The above two publications confirm the feasibility of repeat hepatectomy for recurrent HCC.

Concerning the survival rate after hepatectomy for recurrent HCC, data in the literature confirm the long-term survival of patients after this surgical treatment (Table 1). In the review by Chan *et al*[15], the 5-year survival rate was evaluated at between 22% and 83% with a median rate of 52%. Mise *et al*[16] found a 5-year overall survival rate after second liver resection of 60.5% and after third hepatectomy or more of 68.2%. They reported no significant difference between first, second and third or more hepatectomy for overall survival rate. Yamashita etal showed a 5-year overall survival rate of 60% after second and 43% after third or more hepatectomy[17].

Second resection or more for recurrent HCC enabled long-term survival and must be considered in cases of resectable liver intrahepatic recurrence of HCC.

So, second resection or more could be safely performed without high morbidity or mortality and allow prolonged overall survival, but what about disease free survival thereafter?

According to the review published by Chan *et al*[15], length of median disease free survival was 15 mo, ranging between 7 and 32 mo. In the more recent articles by Mise *et al*[16] and Yamashita *et al*[17], the 5-year disease free survival rate was 17.9% and 29% after second hepatectomy and 12.8% and 18% after third hepatectomy or more respectively.

In our personal experience in an intention-to-treat study comparing the results of repeat resection with local ablation of recurrent HCC (data not published), we found a 5-year overall survival rate of 27% after repeat hepatectomy.

In conclusion, repeat hepatectomy is a feasible treatment for recurrent HCC and should be considered for patients with one HCC nodule and good liver function with sufficient estimated liver remnant. It enables long term overall survival even in cases of third or more repeat hepatectomy.

THE ROLE OF LIVER TRANSPLANTION

The advantage of liver transplantation after first hepatic recurrence is to treat the underlying cirrhotic liver to prevent another recurrence.

SLT

In cases of recurrent HCC according to Milan criteria, SLT could be proposed if age of patients and comorbidity allow.

In 2000, Majno *et al*[18] were the first to describe SLT for recurrent HCC in selected patients and showed that overall survival and disease free survival were the same after primary liver transplantation (PLT) or SLT. A meta-analysis by Zhu *et al*[19] of 14 studies conducted between 2000 and 2012 confirmed that SLT offers the same mortality rate as PLT. The mortality rate of ten studies pooled was 6.34%, with no significant statistical

Table 2 Five years overall survival and prognostic factors for overall survival after salvage liver transplantation

Ref.	Type of study	Patients (*n*)	5 yr overall survival	Prognostic factors for overall survival
Sapisochin *et al*[24]	Case control study	17	52%	
Wu *et al*[28]	Retrospective study	36	69.4%	
Liu *et al*[29]	Retrospective study	39	61%	
Fuks *et al*[11]	Retrospective study	138	71%	
Guerrini *et al*[22]	Retrospective study	28	49.2%	
Chan *et al*[20]	Systematic review	319	62%	
Qu *et al*[30]	Retrospective Study	111	49.5%	Edmonson grade Hepatic vein invasion Portal vein invasion TNM stage
Lee *et al*[31]	Retrospective study	69	54.6%	Time to recurrence Alpha foetoprotein > 200 ng/mL HCC outside Milan criteria

HCC: Hepatocellular carcinoma.

difference with PLT.

Another systematic review published by Chan *et al*[20] in 2013, showed a median perioperative mortality rate of 5% (0%-24%).

Regarding perioperative morbidity, no significant statistical difference was found compared to PLT. The morbidity rate reached 34% in the meta analysis by Zhu *et al*[19].

Laurent *et al*[21] showed that SLT could be safely performed after open primary liver resection or laparoscopic liver resection but that laparoscopic liver resection required less operative time, and blood transfusion.

All these data confirm that SLT could be safely performed without high morbidity or mortality rate.

The short and long-term outcomes after SLT have been studied to evaluate the validity of this therapeutic strategy (Table 2).

Guerrini *et al*[22] published a 5-year overall survival rate of 49.2%. The meta analysis by Zhu *et al*[19] showed that the 5-year overall survival rate was lower after SLT than after PLT. In the review by Chan *et al*[20], the median overall survival rate reached 62% at 5 years with a range between 41% and 89%.

Another point to highlight regarding SLT strategy is the disease free survival rate. In the literature, the median 5-year disease free survival rate was 67% with a range between 29% and 100%[20].

Even if no prospective studies have been published on SLT, the literature confirms the feasibility of SLT that could achieve long-term survival and prolonged disease free survival after primary resection of HCC.

INDICATIONS FOR REPEAT HEPATECTOMY OR SLT

There are no current guidelines on treatment of choice for recurrent intrahepatic HCC. If recurrence is beyond Milan criteria, SLT cannot be performed. If intrahepatic recurrence is within Milan criteria with age 70 years or less, no medical contra indications, portal hypertension and/or abdnormal bilirubin, patients could be proposed liver transplantation. Because of the lack of liver donors, some authors suggest identifying criteria for high or low risk of recurrence to choose between SLT or repeat resection in patients within Milan criteria for recurrent HCC.

The delay of recurrence seems to have an impact on the long-term outcomes of treatment. In a single center study, Huang *et al*[23] showed that overall survival rate was significantly better after repeat hepatectomy in patients with late recurrence than in patients with early recurrence. In this study, the cut-off was 18 mo. Furthermore, in the review by Chan *et al*[15], the only prognostic factor, which impacted on the outcome after repeat hepatectomy, was time to recurrence. A time to recurrence greater than 12-18 mo allowed better long-term survival.

From data in the literature, we should consider repeat hepatectomy in patients with a time to recurrence of more than 12 mo even if this cut-off is arbitrary.

Sapisochin *et al*[24] published a comparative study to determine if patients eligible for SLT should be contraindicated because of high risk of recurrence. In this article, they showed that patients with early recurrence after liver resection for primary HCC had statistically significant poor outcomes after salvage transplantation, especially in patients with poorly differentiated tumors. Even if this study included a small number of patients, it suggests that SLT should be limited in these cases.

In 2012, Belghiti *et al*[10] identified predictive factors for nontransplantability because of recurrence beyond Milan criteria: microvascular invasion, satellite nodules, tumor size > 3 cm, poorly differentiated tumors and liver cirrhosis. In the presence of three factors or more, Belghiti *et al*[10] proposed performing liver transplantation prior to recurrence to avoid recurrence beyond Milan criteria. For patients with fewer than three negative factors, liver transplantation should be performed only in cases of HCC recurrence.

Another publication by Lee *et al*[25] confirmed that early recurrence (before 8 mo) after primary liver

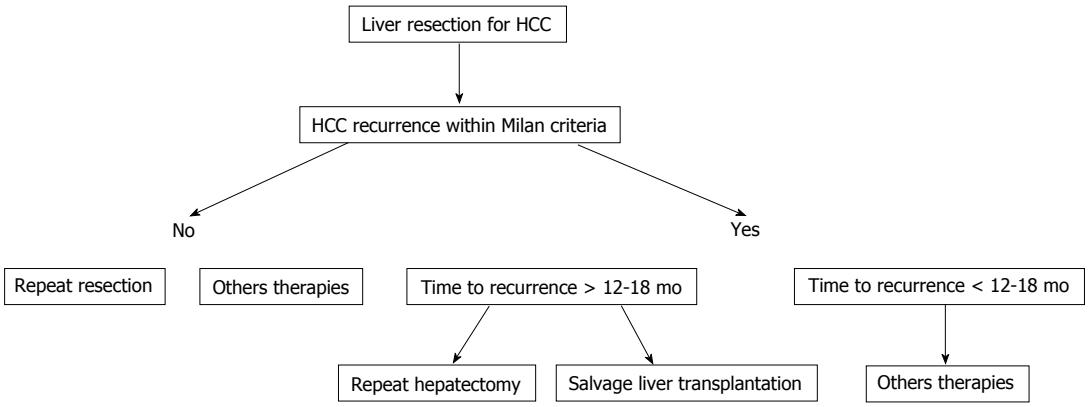

Figure 1 Proposed decisional algorithm according to delay of recurrence. HCC: Hepatocellular carcinoma.

resection was a negative factor for disease free survival after SLT. A rate of 200 ng/mL or more and recurrence beyond Milan criteria were also independent negative prognostic factors for disease free survival after SLT. In this study, patients with one or more of these factors showed worse overall survival and poorer disease free survival than patients without these three risk factors.

Due to insufficient data in the literature and absence of prospective studies, it is difficult to propose a decisional algorithm (Figure 1). We should consider that time to recurrence is the most important prognostic factor for impairment of overall survival after repeat hepatectomy or SLT. In cases of HCC recurrence before 12-18 mo, we should not perform SLT or repeat hepatectomy and other therapies such as chemoembolization or sorafenib should be considered. The role of "prophylactic" liver transplantation must be specified, especially in patients with negative histological factors so to avoid recurrence beyond Milan criteria.

In conclusion, there are no standardized guidelines for the therapeutic strategy of intrahepatic HCC. We should consider repeat hepatectomy and SLT as safe and feasible treatments. These two treatments allow long-term outcomes. Nevertheless, the place of SLT remains to be clearly defined. According to data in the literature, certain negative histological factors as well as delay of recurrence should be taken into account when choosing the best treatment for the patient. A prospective study evaluating SLT and repeat hepatectomy is warranted to confirm the place of liver transplantation after primary resection of HCC and prior to recurrence.

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MINIREVIEWS

Drug- and herb-induced liver injury: Progress, current challenges and emerging signals of post-marketing risk

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Abstract

Drug-induced liver injury (DILI) and herb-induced liver injury is a hot topic for clinicians, academia, drug companies and regulators, as shown by the steadily increasing number of publications in the past 15 years. This review will first provide clues for clinicians to suspect idiosyncratic (unpredictable) DILI and succeed in diagnosis. Causality assessment remains challenging and requires careful medical history as well as awareness of multifaceted aspects, especially for herbs. Drug discontinuation and therapy reconciliation remain the mainstay in patent's management to minimize occurrence of acute liver failure. The second section will address novel agents associated with liver injury in 2014 (referred to as "signals"), especially in terms of clinical, research and drug development implications. Insights will be provided into recent trends by highlighting the contribution of different post-marketing data, especially registries and spontaneous reporting systems. This literature scrutiny suggests: (1) the importance of post-marketing databases as tools of clinical evidence to detect signals of DILI risk; and (2) the need for joining efforts in improving predictivity of pre-clinical assays, continuing post-marketing surveillance and design *ad hoc* post-authorization safety studies. In this context, ongoing European/United States research consortia and novel pharmaco-epidemiological tools (*e.g.*, specialist prescription event monitoring) will support innovation in this field. Direct oral anticoagulants and herbal/dietary supplements appear as key research priorities.

Key words: Hepatotoxicity; Liver damage; Herb; Signal; Safety; Predictivity

Core tip: Drug- and herb-induced liver injury remains a clinical challenge, attracting multidisciplinary interest for its translational aspects (from bench to bedside approach and vice versa). When considering differential diagnosis in patients with liver damage, clinicians should always keep in mind drugs and herbs as possible liver offenders, especially in subjects with comorbidities requiring long-term multiple therapies (likelihood of drug interactions). Drug withdrawal and therapy reconciliation

represent key issues in patient' management to minimize the risk of acute liver failure. Notwithstanding the progress in the tools for early detection of hepatotoxicity, there is growing literature on drugs and herbs possibly associated with liver injury in the post-marketing phase: often undetected during drug development, signals of liver toxicity emerge from spontaneous reporting systems and registries. This calls for a joint, multi-disciplinary action to improve predictivity of pre-clinical assays, continuing post-marketing surveillance and designing *ad hoc* population-based studies.

Raschi E, De Ponti F. Drug- and herb-induced liver injury: Progress, current challenges and emerging signals of post-marketing risk. *World J Hepatol* 2015; 7(13): 1761-1771 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i13/1761.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i13.1761

INTRODUCTION: A MULTIDISCIPLINARY FIELD OF INTEREST

Drug- and herb-induced liver injury (DILI and HILI, respectively) continues to attract interest, as shown by the growing number of publications indexed in PubMed. A broad strategy (*i.e.*, by combining the terms DILI, drug-induced liver injury/damage, HILI/damage, hepatotoxicity), from January 1st to December 31st 2014, yielded 1060 publications, with a mean publication rate of 1440 articles in the last 5-year period (search performed on Jan 13th, 2015) (Figure 1). A publication trend can be easily found in the last 15 years, with remarkable increase since 2010, especially for pre-clinical evidence; as compared to 2013, the apparent decrease in the overall number of publications in 2014 is likely to be related to the delay in publication indexing rather than to an actual decrease in publication rate.

The multidisciplinary interest is indicated by the variety of periodicals covering this topic in 2014: apart from dedicated high-ranking Journals (*e.g.*, *Seminars in Liver Disease*, which entirely devoted an issue to hepatotoxicity, *Gastroenterology*, *Hepatology*, *Journal of Hepatology* and *Clinical Gastroenterology and Hepatology*), also non-specialized Journals published original research articles, expert opinion and comprehensive reviews for primary care clinicians, who frequently encounter this clinical problem in their daily practice[1,2]. The official Journal of the International Society of Pharmacovigilance *Drug Safety* published a supplement, called "Liver Safety Assessment in Clinical Drug Development: A Best Practices Workshop report", describing major achievements and accomplishments for the future (see below for details)[3].

The multifaceted aspects of DILI and its idiosyncratic nature (*i.e.*, unpredictable from the mechanism of drug action) pose a challenge to hepatologists, pharmacologists, toxicologists, clinical investigators and regulators.

From a drug development perspective, DILI caused a number of regulatory actions in the past decades. Very recently, Wang *et al*[4] reviewed formal reasons for non-approval of 27 drug applications and identified hepatotoxicity in 4 (15%) cases. The oral anticoagulant ximelgatran is a typical example of interruption of drug development for hepatic concern: in 2006, the manufacturer withdrew a pending application to the Food and Drug Administration (FDA). Shah[5] analyzed 38 drugs withdrawn between 1990 and 2006, and found that 14 of them (37%) were removed from the market due to hepatotoxicity. A more recent review highlighted that, among 25 safety-based withdrawals in Europe and United States, ten (40%) were related to cardiovascular issues and seven (28%) to gastrointestinal, primarily hepatic, adverse events, which were not predicted from known pharmacological action[6]. From a historical perspective, in several circumstances, hepatotoxic agents were identified after being used in clinical practice for several months: for instance, this was the case of troglitazone, which was withdrawn after more than 3 years on the United States market.

Form a clinical standpoint, the assessment of DILI risk in individual patients should be performed on a case-by-case basis according to different clinical elements (see below), whereas the evaluation of drug-related liver risk in a population perspective requires integration of data originated from multiple lines of evidence and data sources, including clinical trials, observational studies (cohort and case-control approaches), registries, spontaneous reporting systems, case series/reports[7].

We present this overview to highlight present trends and potential new areas of research: because of the large number of studies in the past year and the non-systematic nature of this review, we selected only articles that, in our opinion, provide key contributions to understand the way forward. After a brief description of key aspects to diagnose and manage drug-related liver disease, the next sections are organized by data source and mainly discuss novel agents associated with DILI in various settings. Specifically, the term "signal" will be used thereafter to indicate any new information/data regarding a possible drug-related association with liver damage (either clinical or statistical), which requires further investigation.

DILI AND HILI: CLUES FOR CLINICIANS

Epidemiology

The list of drugs that have been associated with hepatotoxicity is constantly growing[8]. A collaborative study published in 2010 collected information from different sources to select a unified list of drugs associated with DILI: among 385 agents, 319 compounds were identified in three DILI registries (Spain, Sweden and United States), with notable differences among the different cohorts, depending on the drug marketing access and prescribing patterns[9].

Determining the true incidence of DILI remains

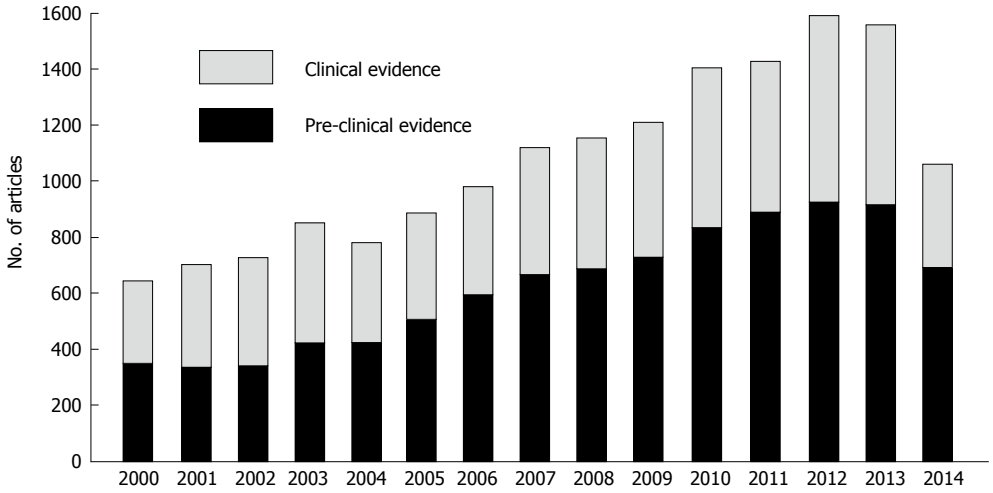

Figure 1 Publication trend over the past 15 years of articles on drug- and herb-induced liver injury, classified in terms of preclinical and clinical evidence. The search was performed in PubMed on January 13th, 2015, through automatic filters and keywords (see text for details).

difficult. The recent population-based study from Iceland found an incidence of 19 cases per 100000 per year[10], higher than previous findings from France and United Kingdom: 13.9 and 2.4 per 100000, respectively[11,12]. The latest retrospective cohort study, using data from the Kaiser Permanente Northern California healthcare system, calculated an incidence rate of any definite drug-induced acute liver failure (ALF) of 1.61 events/1000000 person-years[13]. For a comprehensive discussion of the current status on epidemiology the reader should refer to Björnsson[14]; in summary, current data consistently identify particular drugs (*e.g.*, amoxicillin-clavulanic acid, isoniazid) and confirms the two drug classes of antibiotics and antiepileptics as most prevalent in causing hepatotoxicity. Notably, recent population-based epidemiologic data on acute liver injury (ALI) found that incidence of ALI in pediatrics is relatively low and broadly comparable with adults, with higher incidence rate in Italy, as compared to the Netherlands (73 *vs* 21/100000 person-years, respectively) and antibiotics as the drugs most frequently implicated with ALI[15].

As regards HILI, the absence of regulatory guidelines further compromises calculation of true incidence. Notably, complementary and alternative medicines was one of the two most common etiologies reported among 24112 Chinese patients with DILI[16]. Current estimates suggest that 15% of DILI are caused by herbs and a recent tabular compilation of published case reports, including traditional Chinese medicines, established causality for 28 out of 57 different herbs and herbal mixture selected in 77 publications[17].

Risk factors and pathogenesis

The pathogenesis of DILI and HILI is only partially understood, with three intertwined factors: (1) Clinical host-related risk factors. Age and gender are perceived as non-modifiable risk factors[18]; recent studies highlighted age- and gender-related differences in the reporting of DILI that depend on drug and/or drug class (*e.g.*, male were overrepresented in cases associated with antivirals for systemic use, whereas ALF and hepatocellular injury were more frequently reported among children)[19,20]. There is considerable body of evidence (through genome-wide association studies) indicating that susceptibility to DILI is genetically determined, at least for some compounds (*e.g.*, flucloxacillin)[21]; (2) Environment. Our understanding of environmental factors is limited, with coffee, alcohol consumption, and diet that have not been identified as *bona fide* risk factors for DILI; and (3) Drug-related risk factors. Recent studies have suggested that drugs with high daily dose (> 50 to 100 mg/die), high lipophilicity (known as the "rule-of-two") and extensive hepatic metabolism are more prone to cause DILI[22,23]. The so-called "damage hypothesis" regards the inadvertent generation of reactive metabolites or parent drug-protein complex that can directly or indirectly mediate intracellular damage *via* oxidative endoplasmic reticulum stress, mitochondrial damage, inhibition of bile salt export pump. In the "hapten hypothesis", the drug-protein or metabolite-protein adduct leads to inadvertent activation of the adaptive immune system[24]. At the current state of the art, however, the actual clinical relevance of these pathophysiological mechanisms still requires formal evaluation.

Diagnosis

Patients with DILI pose substantial diagnostic, prognostic, and therapeutic challenges to the gastroenterologist[25]. The presentation of DILI may vary from asymptomatic liver enzyme elevation (which incidentally may come to the attention of clinicians during planned laboratory tests for other medical reasons) to ALF causing hospital admission and potentially requiring transplantation. The thresholds and cutoffs for enzymes elevation has been subject to debate and changes over time for a number of reasons. From one hand, the prevalence of non alcoholic fatty liver disease (NAFLD) is increasing and some subjects are known as "adaptors" (showing transient increase in enzyme levels, which eventually

return to baseline despite continuation of the drug); on the other hand, it is crucial to identify early signals of DILI that are predictive of ALF during drug development[26]. Currently, a 3- to 5-fold elevation (x upper limit of normal) in alanine aminotransferase or aspartate aminotransferase represent the most commonly used thresholds. In most of the cases, DILI resolves following drug discontinuation, albeit up to 20% of patients progress to chronic liver damage further challenging the clinicians' management skills. Although usually the first step in describing DILI is to differentiate "idiosyncratic" (unpredictable) from intrinsic (predictable) type, this distinction is highly debated and, more importantly, it does not affect clinical management. Therefore, diagnosis of DILI first and mostly depends on obtaining a detailed patient's history and thoughtful use of diagnostic tests[25].

Overall, the clinical assessment focuses on four major areas: (1) timing (exposure or latency; recovery or dechallenge; information about the latest laboratory test before starting treatment can be of great value); (2) pattern of liver biochemistries at presentation (this aspect may influence the request for serological, imaging investigation and liver biopsy); (3) hepatotoxicity profile of suspect agent (some drugs such as telithromycin may have a distinctive clinical signature that may be indicative of high mortality rate[27]); (4) other extra-hepatic signs/symptoms (immune-allergic features such as rash, fever and eosinophilia argue for a drug etiology); and (5) exclusion of competing causes: in particular, acute viral hepatitis caused by less common viruses (type E hepatitis virus, cytomegalovirus, Epstein-Barr virus) and chronic liver diseases (*e.g.*, NAFLD) should be ruled out. Judicious use of blood tests and liver imaging are necessary, but liver biopsy, while often helpful, is not mandatory.

Different clinical algorithms have been published to facilitate diagnosis and management; the reader can refer to the latest recommendations provided by the American College of Gastroenterology[28]. These diagnostic algorithms are based on clinical scoring systems. According to major experts in the field, the Council for International Organizations of Medical Sciences scale has the potential to be a standard scale for DILI and HILI causality assessment and can be adopted by physicians, regulatory agencies, expert panels and the scientific community. Other advantages include its liver specificity and its validation for hepatotoxicity cases, with excellent sensitivity, specificity and predictive validity based on results obtained from cases with a positive re-exposure test[29]. In case of suspect, instead of checking published case reports (which are of varying quality), clinicians should refer to the *LiverTox* database (http://www.livertox.nih.gov/), a free periodically updated online DILI resource detailing information on more than 600 agents. It should be kept in mind that, causality assessment and actual diagnosis is based on a case-by-case clinical judgment and, in doubtful cases, expert consultation is needed[28,30].

Two clinical issues are particularly challenging for gastroenterologists: HILI and drug-induced autoimmune liver disease. The former may have similar or identical clinical presentation of DILI, raising debate on whether or not HILI needs a separate term. However, major differences exist between DILI and HILI: DILI is usually caused by a single drug (either chemical or biological), whereas HILI is triggered by a chemical mixture from the herbal extract, which often lacks regulatory assessment and surveillance. Herbal product quality varies and is a major issue in HILI, adding to the complexity in evaluating causality for herbs. This may explain why HILI is considered as a poorly defined entity, is a neglected disease, and requires special attention[31]. The latter (drug-induced autoimmune liver disease) is emerging as a poorly defined under-reported and underestimated liver disorder, and poses particular diagnostic dilemma[32]; indeed, overlaps and different clinical scenarios exist among DILI and autoimmune hepatitis. What is clear is that the diagnosis of autoimmune hepatitis is often made in the setting of a patient under poly-pharmacotherapy. Discriminating between true autoimmune hepatitis triggered by drugs and immune-mediated DILI still remains a challenge.

Management

The key treatment of DILI remains withdrawal of the offending medication[28] (hence, the importance of correct differential diagnosis). However, early drug discontinuation does not always prevent the occurrence of ALF. Nonetheless, only a small fraction (10%) of idiosyncratic DILI exceeds in ALF, with coagulopathy and any degree of encephalopathy. Unfortunately, prognostic scores to early predict the clinical outcome for DILI reaching the threshold of ALF are still under development. From a drug development standpoint, the decision on drug discontinuation should be carefully balanced, with stopping rules suggested by the FDA[33].

No definitive therapies are available for idiosyncratic DILI with or without ALF: N-acetylcysteine (NAC), the antidote for acetaminophen overdoses (dose-dependent DILI), may be considered in adults with early-stage ALF, for its good safety profile and some evidence for efficacy in early coma stage patients. A meta-analysis (4 trials selected) concluded that NAC is safe for non-acetaminophen-induced ALF. It can prolong patients' survival with native liver without transplantation and survival after transplantation, without improvement in the overall survival[34]. Re-exposure to a drug that is thought likely to have caused hepatotoxicity is strongly discouraged, especially if the initial liver injury was associated with remarkable enzyme elevation. Follow-up is also needed until resolution, as chronicity may occur in approximately 14% of those experiencing DILI[28].

SIGNALS EMERGING FROM SPONTANEOUS REPORTING SYSTEMS AND CASE SERIES/REPORTS

Because of limited predictive value of pre-clinical

assays[35], the lack of fully validated biomarkers and the limited power of pre-marketing randomized clinical trials to detect rare safety issues, large spontaneous reporting systems of adverse drug reactions are a pivotal source for early identification of safety signals, especially for rare idiosyncratic events such as DILI. Several analyses on spontaneous reporting databases have been published in 2014 (60 out of 369 publications retrieved in PubMed are based on case reports/series and pharmacovigilance databases), thus highlighting the contribution of this tool as a source of clinical evidence. For this reason, we provide here below a few key examples.

Three pharmacological classes of medications were investigated through pharmacovigilance databases: *direct oral anticoagulants* (DOACs), antimycotics and antidepressants. As regards DOACs, although a recent systematic review on phase Ⅲ randomized clinical trials failed to demonstrate a significant risk of DILI[36], the experience gained from the history of ximelagatran suggested that caution is needed before considering them free from DILI risk. As a matter of fact, case series have become to accrue and suggested a potential safety signal, especially for rivaroxaban[37,38]. In particular, the assessment of spontaneous reports submitted to the publicly available FDA Adverse Event Reporting System (FAERS) detected a disproportionality signal of DILI for rivaroxaban (including ALF), whereas no association emerged for dabigatran, even when potential competition biases were tested. Notably, a considerable proportion of DILI reports of rivaroxaban (42%) and dabigatran (37%) co-listed possible hepatotoxic and/or interacting drugs, with fatal outcome and very rapid time-to-onset in almost half of ALF reports[39]. These signals should not automatically generate alarm, but certainly prompt comparative population-based studies to characterize and quantify the actual risk, taking into account drug- and patient-related risk factors[40]. Meanwhile, as DILI is unpredictable, these findings strengthen the importance of timely pharmacovigilance to detect post-marketing signals of DILI and underline the role of clinicians in early recognition of signs/symptoms suggestive of severe hepatic damage.

Among the first detected hepatotoxins in 2014, we selected the first post-marketing report by pomalidomide, the latest immunomodulating drugs approved by the FDA for multiple myeloma. The causal association was based on the temporal association with drug exposure and the exclusion of other causes[41]. Notably, DILI occurred despite dose titration and monitoring of liver function. A second biopsy was performed because, within 2 wk of completing steroids, bilirubin markedly increased; this second histological evaluation raised the possibility of acute hepatitis presentation of chronic graft-*vs*-host disease. Steroids should be considered if hepatotoxicity persists despite discontinuation of pomalidomide.

A case series from Germany highlighted a typical clinical pattern of flupirtine: it almost exclusively occurred in females and was characterized by hepatocellular pattern as a key histological feature and clinical manifestation with jaundice and ALF. In March 2013, the European Medicines Agency (EMA) recommended to limit the duration of flupirtine treatment to 2 wk; however, data by Douros *et al*[42] suggested that early, severe hepatotoxic symptoms cannot be ruled out.

As regards *antimycotics,* the 2013 regulatory interventions on ketoconazole for DILI (the oral formulation was withdrawn) posed a prescribing challenge to clinicians, who should now carefully consider safer therapeutic alternatives. Data mining of the publicly available FAERS database highlighted that antimycotics are involved in approximately 3% of DILI cases (including ALF events); as compared to topical-administered antimycotics, virtually all systemic antimycotics (including ketoconazole, newer triazole derivatives voriconazole and posaconazole, as well as terbinafine) generated a significant disproportionality, indicating a post-marketing signal of risk. Thus, clinicians should assume a potential class effect and, in case a therapeutic switch is considered, careful monitoring is recommended, especially in critical poly-treated patients with multiple comorbidities[43]. The worldwide re-appraisal of oral ketoconazole reminds clinicians of the importance of liver safety during oral antifungal treatment and carries implications for future antifungal development[44]. In fact, clinical research on drug-drug interactions is now challenged by the prohibition of ketoconazole, previously used as a prototype CYP3A4 inhibitor in healthy volunteers. Ritonavir and itraconazole have been suggested as possible alternatives, but not clarithromycin[45].

The case of *antidepressants* carries similar clinical implications. A review of clinical data suggested that duloxetine, bupropion, trazodone, tianeptine, and agomelatine are associated with greater risk, as compared to selective serotonin reuptake inhibitors[46]. Although an infrequent event, DILI from antidepressants may be irreversible, and clinicians should be aware of this. Data from Spanish, French, and Italian spontaneous reporting databases consistently showed a signal of hepatotoxicity for agomelatine[47]. These data and other clinical evidence prompted assessment by the EMA, which confirmed the positive risk-benefit profile of agomelatine, although measures for intense liver function monitoring and new contraindications were introduced: elevation of liver enzymes higher than 3 times as compared to reference values, hepatic impairment (not further specified), parallel use of potent CYP1A2 inhibitors[48].

SIGNALS EMERGING FROM REGISTRIES

During the last decade, data from large registries of DILI patients have been published. Although most of these registries cannot be formally considered population based, they do provide important data on relative prevalence of agents, they may better detect rare hepatoxicity signals in early post-marketing phase and also allow important comparisons between countries[10,14,49].

In 2014, the United States DILI Network (DILIN) continued to publish new analyses from prospective registry, especially by defining clinical signatures of specific agent. Notably, a new syndrome was identified and characterized after a single intravenous dose of cefazolin: 1-3 wk of latency period after exposure (usually following a minor outpatient surgical procedure), marked cholestasis and a self-limited moderate to severe clinical course[50].

New information was provided also for anti-tumor necrosis factor (*TNF*) agents, among which infliximab was the most frequently implicated (1 in 120 patients): 50% of patients required steroid therapy, but without long-term treatment. Moreover, the addition of methotrexate to anti-TNF therapy might reduce the risk of DILI[51].

The DILIN consortium also addressed the spectrum of statins hepatotoxicity and provided novel previously unknown aspects: DILI with statins is rare and characterized by variable patterns of injury, a range of latencies to onset, autoimmune features in some cases, and persistent or chronic injury in 18% of patients, with an autoimmune phenotype in most of the cases[52].

Herbal and dietary supplements (HDSs) were also under scrutiny[53]. During the 2004-2013 period, it was noted that the proportion of liver injuries attributed to HDSs increased from 7% to 20%, as compared to medications. It is noteworthy that, bodybuilding HDSs are the most commonly implicated class of products and, most importantly, non-bodybuilding HDSs (*e.g.*, products for weight loss) can cause more severe liver injury than conventional medications, as reflected by a higher transplantation rate (13% *vs* 3%). As discussed below, the relationship between herbal administration and hepatic safety represents a current research question, as demonstrated by some products used to treat liver disease that may also have a detrimental hepatic effect and confounders exist (*e.g.*, multiple ingredients and sometimes undeclared components)[54].

SIGNALS EMERGING FROM OBSERVATIONAL STUDIES

Observational studies, namely population-based case-control and cohort studies, represent key pharmaco-epidemiological tools aimed to assess the likelihood of association. These studies are usually triggered by previous (early) analysis on spontaneous reporting systems highlighting possible drug-event association (signal detection). These analytical approaches may allow risk quantification of adverse events that have a long delay between exposure and clinical manifestations, highlight new risks associated with old drugs, as well as adverse events characterized by high background incidence rates and less likely to carry a drug-induced component[6].

Although these post-marketing studies are highly representative of actual practice (*i.e.*, high external validity as compared to clinical trials), methodological complexity and the need for long-term follow up (for cohort studies) often compromise feasibility and optimal data collection.

In 2014, we identified three publications deserving consideration. The first one regards a comparative hepatic evaluation of antithyroid drugs, for which only anecdotic case reports/series were provided: in a population-based cohort study on Taiwan National Health Insurance Research Database, methimazole/carbimazole showed a dose-dependent increased risk of hepatitis, as compared to propylthiouracil, while the risks were similar for ALF and cholestasis[55].

The second contribution emerged from the hospital-based Berlin Case-Control Surveillance Study: apart from known hepatotoxic drugs (*e.g.*, amiodarone), novel hepatotoxic risk was suggested for biperiden, thus highlighting the need for post-authorization safety studies[56]. These types of researches should be replicated in different settings to highlight possible differences in the pattern of drug use among the different clinical scenarios.

The third study was conducted within the Exploring and Understanding Adverse Drug Reactions by Integrative Mining of Clinical Records and Biomedical Knowledge project and designed as a multi-country cohort study in 7 European healthcare databases with a focus on ALI in children and adolescents. Apart from known signals, three associations (*i.e.*, domperidone, flunisolide and human insulin) were previously undocumented (literature and labels) either in adults or in children, whereas two drugs (citalopram, cetirizine) were not previously described in children but reported in adults[57].

SIGNALS EMERGING FROM CLINICAL TRIALS (INCLUDING META-ANALYSES)

Apart from cardiotoxicity, DILI is a recently recognized safety concern, albeit not formally quantified yet, of oral tyrosine kinase inhibitors (TKIs). The latest meta-analysis of 6 randomized controlled trials on anti-angiogenic TKIs found that hepatotoxicity is a relatively common (occurring in 23%-40% of patients) but non severe event (only 5% of patients experienced high-grade toxicity)[58]. These data corroborate previous findings from an earlier meta-analysis[59] and indicate that TKIs are associated with potentially fatal hepatotoxicity, usually reversible on dose reduction or drug discontinuation. Of note, incidence varies widely among agents, thus suggesting that a class effect is unlikely: the potential for serious hepatotoxicity with lapatinib, pazopanib, ponatinib, regorafenib and sunitinib was believed to be sufficiently high as to require a boxed label warning. Post-marketing surveillance is warranted, especially for newer agents, to assess the actual role of TKIs in the occurrence of DILI, especially in the presence of hepatocellular carcinoma, hepatic metastasis and potential drug interactions[60]. Pre-clinical research should investigate the mechanism of TKI-related DILI; the

formation of reactive metabolites has been suggested to play a role in the pathogenesis, at least as a key prerequisite. Current clinical management strategies are based on (1) switching to an alternative TKI with similar mechanism of action (*e.g.*, erlotinib *vs* gefitinib); (2) using an alternative dosing regimen (reduced doses or dosing frequency); and (3) introduction of steroids for the treatment and prevention of hepatotoxicity (if autoimmune response is present)[61].

Very recently, the debate on alogliptin hepatotoxicity has aroused interest; Scheen reviewed the pharmacokinetics and hepatic profile of incretin-based therapies and concluded that the overall liver safety of dipeptidyl peptidase-4 inhibitors is reassuring, and, in particular, that "no hepatotoxicity has been reported in the development programme of alogliptin"[62]. By contrast, Barbehenn *et al*[63] pointed out the numerical imbalance, albeit not statistical significant, emerging for alogliptin from the publication of the Examination of Cardiovascular Outcomes with Alogliptin *vs* Standard of Care (EXAMINE) trial. This signal together with other reports from Japan prompted a careful FDA hepatological assessment, which culminated in recommending liver function evaluation before starting alogliptin therapy. Scheen's rebuttal challenged the FDA measures by providing new pooled data that "indicate that alogliptin is associated with a low risk of hepatic toxicity"[64]. Considering the heterogeneous marketing life, penetration and utilization of the different dipeptidyl peptidase-4 inhibitors, analytical post-marketing studies should be encouraged, especially in the wake of a recent FAERS analysis, showing no signal of liver injury for alogliptin, but statistically significant associations for sitagliptin, saxagliptin and vildagliptin[65].

PERSPECTIVES

The role of biomarkers in drug development

The present multidisciplinary interest in DILI and HILI is well documented by the exponential increase in pre-clinical publications, which suggest a gap in knowledge on the predictivity of *in vitro/in vivo* assays.

Despite intensive efforts to develop biomarkers sufficiently predictive of DILI risk in earlier phases of drug development, there is still room for improvement in this area as no biomarkers are currently validated for routine use[66]. The role of new serum biomarkers such as glutamate dehydrogenase, high mobility group box protein 1, and microRNA-122 is under scrutiny for possible use in diagnosis and prognosis and provide important insights into the mechanisms of the pathogenesis, which is only partially understood (from bench to bedside)[24]. Thus, new prediction methodologies are needed.

Emerging issues in pre-clinical research

The role of animal studies remains questionable, mainly because of the incomplete understanding of the mechanisms underlying DILI, as well as marked species differences in response to, and in the metabolism of, xenobiotics. As a result, there is currently no universally accepted animal model and no formal approval is granted by Regulatory Agencies.

The use of various techniques involving liver cell cultures for DILI prediction is highly controversial for several reasons. Although it is well accepted the contribution of drug metabolism as initiating step, numerous mechanistic studies have emphasized the fact that DILI may be a multicellular event. Over the past decade, attempts have been made to compile hepatotoxicity data and develop *in silico* models to be used as a first-line screening of drug candidates[67]. *In vitro* battery for hepatotoxicity testing comprises a number of cell models, among which 3D cultures, engineered liver-derived cell line and pluripotent stem cell-derived hepatocytes are emerging as promising for toxicological screening of drug candidates[68]. In this context, a chimeric TK-NOG mice model with humanized livers was recently implemented as predictive model to assess cholestatic liver damage induced by fialuridine and bosentan, known to be hepatotoxic from clinical trials. These findings suggested that the use of chimeric mice could improve the pre-clinical drug safety assessment of candidate drugs[69,70].

Novel and current clinical issues

From a clinical standpoint, although progress has been achieved in diagnosis and timely recognition of hepatic damage by drugs and herbs, the idiosyncratic nature of liver toxicity calls for continuing monitoring and vigilance of patients, especially those with comorbidities requiring chronic long-term treatment with multiple agents. In clinical practice, viral hepatitis and NAFLD represent the two most common hepatic disorders that can mimic DILI and should be always considered among the various differential diagnoses. In clinical phases of drug development, DILI prediction and detection relies on Hy's law and the Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) plot[71]. Recently, Robles-Diaz *et al*[72] suggested a new model using liver enzymes to improve prediction of ALF. Moreover, Fontana *et al*[73] analysed 660 patients with definite, highly likely, or probable DILI and found that, within 6 mo of DILI onset, 9.4% of patients either died or required liver transplantation. However, these results do not convincingly demonstrate that mild liver test abnormalities seen during follow-up are of clinical significance[74]. In populations with underlying liver diseases, such as viral hepatitis, liver safety assessment is particularly challenging, especially because liver enzymes elevation at baseline is quite common, as well as administration of concomitant hepatotoxic drugs and comorbidities such as steato-hepatitis and dyslipidemia. In addition, in oncology, hepatic abnormalities may reflect involvement of the liver in tumor progression[75].

As a general recommendation, clinicians should: (1) be aware and consider DILI and HILI among the various differential diagnoses; (2) inform patients of the potential risk associated with certain drugs, if documented; (3) discontinue suspect offender agent(s); (4) start

immunosuppressive agents (*e.g.*, corticosteroids) in case an autoimmune liver disease is considered; (5) overall reconcile drug therapy by paying attention to concomitant medications; (6) consider referral to specialized centers for support in diagnosis and management; and (7) voluntarily report potential drug-related clinical event, especially those with serious life-threatening outcome (*e.g.*, ALF).

Research agenda on HILI

The research agenda of HILI is complicated by a further dimension, as the most effective approach to identify culprit herbal agents requires careful separation of products into their often multiple components, followed by *in vitro/in vivo* toxicological evaluation[53]. In addition, the precise epidemiology is far from being fully appreciated, mainly because the available evidence is mostly based on case reports/series, which have been systematically collected to create tabular lists[17]. Large registries will be crucial for this purpose; in fact, the DILIN consortium created a repository to explore the hepatotoxic potential of certain ingredients (as of October 1st, 2014, 318 herbal products have been collected) and a workshop took place on May 5-6, 2015 to define opportunities and directions for future research[76].

Among cases of HILI reported worldwide, the following products should be emphasized: herbals containing pyrrolizidine alkaloids, herbal medicine as part of traditional chinese medicine, kava, black cohosh and HDSs (*e.g.*, Herbalife®, Hydroxycut, green tea, anabolic steroids). From one hand, the use of these products, mainly for healthy indications such as weight loss and improvement of physical performance, is extensive and largely uncontrolled by regulatory authorities. On the other hand, their safety and efficacy have not been rigorously tested, thus strengthening the importance of active vigilance, international harmonization and regulatory supervision similar to synthetic drugs, especially in the light of modern globalization[77].

Existing projects

The interest in DILI is also underlined by the number of active research consortia worldwide. Apart from the American DILIN, set up in 2003 and now including retrospective and prospective nationwide registries (https://dilin.dcri.duke.edu/), the International Serious Adverse Events Consortium is a pharmaceutical-industry-led and FDA-supported international research network, focused on identifying and validating DNA variants predictive of the risk of drug-induced serious adverse events. Launched in 2007, by the end of 2015 the consortium expects to have aggregated information for 7500 cases, of which 2500 on DILI phenotype[78]. The DILI-sim Initiative, started in early 2011, is a pre-competitive partnership aiming to develop a computational model (DILIsym® software) for early prediction of DILI (http://www.dilisym.com/). Liver Toxicity Knowledge Base is another FDA-supporter project; it was developed to exploit systems biology analysis for DILI assessment and prediction (http://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/default.htm). As a first step, a benchmark dataset of 287 drugs with established DILI risk was created using the FDA-approved prescription drug labels[79].

In Europe, there are two initiatives comprising European Federation of Pharmaceutical Industries and Associations members, Academia, Regulatory Agencies and Small Medium Enterprises: the Innovative Medicines Initiative called Safer and Faster Evidence-based Translation (http://www.imi-safe-t.eu/htdocs/), involving 25 members and aiming at qualifying new safety biomarkers for pre-clinical and clinical regulatory decision-making needs; and Mechanism based integrated systems for the prediction of DILI (http://www.mip-dili.eu/), involving 26 participants, aiming to develop and validate novel *in vitro* assays. The proposal for recent future is to create a Liver Safety Research Consortium comprising representatives from industry, academia and regulatory agencies, a framework similar to the highly successful Cardiac Research Safety Consortium[3].

CONCLUSION

The aforementioned multidisciplinary consortia represent excellent examples to boost innovation and develop collaborative research comprising all stakeholders. The curiosity, expectations and evidence emerging from these multidisciplinary networks are certainly welcome to advance the knowledge on DILI prediction, diagnosis and management.

At the current state of the art, the unpredictable nature of DILI and HILI strongly supports (1) the importance of post-marketing studies to fully characterize the actual liver damage associated with drugs and herbs, in terms of drug- and host-related risk factors (clinical pharmacology perspective) as well as the epidemiological dimension (population perspective); and (2) the timely recognition of signs/symptoms indicative of liver dysfunction by clinicians, who should consider the potential responsibility of drugs/herbs among the differential diagnoses.

In the near future, two key topics should be prioritized for research activities. First, HDSs require better understanding of their actual epidemiological magnitude, which may be achieved by considering international harmonization of their regulatory status. Second, the rapid accrual of clinical evidence on liver injury induced by DOACs calls for well-designed post-authorization safety studies, especially in the light of their potential therapeutic role in a triple antithrombotic therapy after acute coronary syndromes[80]. In this context, specialist prescription event monitoring may be a candidate pharmaco-epidemiological tool to assess the real-world risk in clinical practice and develop proper risk management plans, as recommended by the new pharmacovigilance legislation[81].

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Relevance of ADAMTS13 to liver transplantation and surgery

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Abstract

A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) specifically cleaves unusually-large von Willebrand factor (VWF) multimers under high shear stress, and down-regulates VWF function to form platelet thrombi. Deficiency of plasma ADAMTS13 activity induces a life-threatening systemic disease, termed thrombotic microangiopathy (TMA) including thrombotic thrombocytopenic purpura (TTP). Children with advanced biliary cirrhosis due to congenital biliary atresia sometimes showed pathological features of TMA, with a concomitant decrease of plasma ADAMTS13 activity. Disappearance of their clinical findings of TTP after successful liver transplantation suggested that the liver is a major organ producing plasma ADAMTS13. *In situ* hybridization analysis showed that ADAMTS13 was produced by hepatic stellate cells. Subsequently, it was found that ADADTS13 was not merely responsible to development of TMA and TTP, but also related to some kinds of liver dysfunction after liver transplantation. Ischemia-reperfusion injury and acute rejection in liver transplant recipients were often associated with marked decrease of ADAMTS13 and concomitant formation of unusually large VWF multimers without findings of TMA/TTP. The similar phenomenon was observed also in patients who underwent hepatectomy for liver tumors. Imbalance between ADAMTS13 and VWF in the hepatic sinusoid might cause liver damage due to microcirculatory disturbance. It can be called as "local TTP like mechanism" which plays a crucial role in liver dysfunction after liver transplantation and surgery.

Key words: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13; Thrombocytopenia; Microcirculation; Liver dysfunction; von Willebrand factor; Liver transplantation; Acute rejection; Ischemia-reperfusion injury; Hepatectomy; Liver surgery; Local thrombotic thrombocytopenic purpura like mechanism

Core tip: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) is a cleaving protease of von Willebrand factor (VWF). The deficiency of this molecule is known to cause thrombotic thrombocytopenic purpura (TTP). Recent studies revealed that ADAMTS13 might have functional relevance to pathogenesis of various liver disease separately from the development of TTP. Imbalance between ADAMTS13 and VWF in the hepatic sinusoid might cause liver damage due to microcirculatory disturbance. It can be called as "local TTP like mechanism" which plays a crucial role in liver dysfunction after liver transplantation and surgery including ischemia reperfusion injury and acute rejection.

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INTRODUCTION

The liver produces a variety of coagulation and fibrinolytic proteins, which are essential to create the hemostatic network on a basis of coagulation cascade[1,2]. In contrast, plasma von Willebrand factor (VWF) plays a pivotal role in primary hemostasis by anchoring platelets onto the denuded vascular subendothelial matrices under high shear stress generated in microvasculatures. VWF is produced exclusively in vascular endothelial cells as unusually large VWF multimers (UL-VWFM) and the secreted into circulation. Before secretion, VWF is cleaved into the smaller multimers by a specific plasma protease, termed a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13), at the peptide bond between Tyr1605 and Met1606 within the VWF-A2 domain[3,4]. The lack of ADAMTS13 induces excess activity of UL-VWFM and may results in microcirculatory disturbance with formation of thrombi in microvasculatures.

The *ADAMTS13* gene is located on chromosome 9q34, and the enzyme consists of 1427 amino acid residues with a multi-domain structure[5]. The initial northern blotting studies indicated that ADAMTS13 mRNA is exclusively expressed in the liver[6], and the subsequent immunological studies with *in situ* hybridization analyses revealed that ADAMTS13 is unambiguously produced in hepatic stellate cells (Itoh cells)[7]. However, ADAMTS13 was also identified in platelets[8], vascular endothelial cell[9], and kidney podocytes[10]. Therefore, an important question has been arisen which organ is most responsible for maintaining the plasma levels of ADAMTS13 activity. In this regard, we found that pediatric patients with advanced biliary cirrhosis due to bile duct atresia often showed pathological features resembling to thrombotic microangiopathy (TMA) which shows microangiopathic hemolytic anemia, destructive thrombocytopenia, and organ dysfunction caused by platelet thrombi. Further, these patients usually had a significantly low plasma level of ADAMTS13 activity, and interestingly their clinical and laboratory findings rapidly improved after a successful liver transplantation (our original data). These results strongly suggested that the liver is a major organ producing plasma ADAMTS13. In the absence of ADAMTS13, UL-VWFM released from vascular endothelial cells left uncleaved, which induce platelet hyperagglutination under high shear stress and generate platelet thrombi in organ microcirculation[3,4,11-13], typically shown in thrombotic thrombocytopenic purpura (TTP), a life-threatening generalized disease and a phenotype of common pathological features of TMA.

Subsequently we have reported that the decrease of plasma ADAMTS13 activity correlates with the disease progression of various chronic liver diseases including hepatitis C-associated liver cirrhosis[14], the ischemia-reperfusion injury and acute rejection in liver transplant recipients[15], and hepatic dysfunction after hepatectomy for liver tumors[16]. The hepatic sinusoid is the narrowest vascular structure within the liver and is the principal site of blood flow regulation. The anatomical location of hepatic stellate cells, which embrace the sinusoids, provides a favorable arrangement for sinusoidal constriction, and for control of sinusoidal vascular tone and blood flow[17]. Because of this specific microanatomical environment, it is suspected that hepatic stellate cell is a key player in regulating hepatic sinusoidal blood circulation. From this point of view, this review is showing the dynamics of ADAMTS13 activity and its clinical relevance to the pathogenesis of liver dysfunction after liver transplantation and surgery.

ENDOTHELIAL CELL INJURY AND ADAMTS13 IN CIRRHOTIC LIVER

The mechanism of thrombocytopenia in patients with liver cirrhosis provides suggestion to relevance of ADAMTS13 to development of liver dysfunction due to microcirculatory disturbance. It has been speculated that thrombocytopenia in liver cirrhosis is caused by hypersplenism and impaired synthesis of thrombopoietin in the liver[18,19]. However, Uemura *et al*[14,20] provided an evidence of increase of UL-VWFM in patients with severe liver cirrhosis. Thrombocytopenia may be enhanced by platelet aggregation increased UL-VWFM

under high shear stress. Their data showed a significant reduction of plasma ADAMTS13 activity in patients with advanced liver cirrhosis mainly caused by hepatitis C virus infection[20]. The results are consistent with reports by Mannucci *et al*[21] and Feys *et al*[22]. Severity of decreased ADAMTS13 activity was parallel to impaired hepatic functional reserve[20]. The plasma ADAMTS13 activity in Child-Pugh classification (Child) C patients was significantly lower than those in patients with Child A and B. Among these, UL-VWFM-positive patients showed the lowest plasma ADAMTS13 activity, most impaired liver and renal function, and lowest Child-Pugh scores. These results indicate that severe cirrhosis may be prone to platelet aggregation. High susceptibility to thrombotic formation may be supported by high incidence of portal or hepatic venous thrombosis in patients with severe liver cirrhosis[23,24]. Even in the absence of clinically overt thrombotic events, microcirculation may be disturbed by formation of platelet microthrombi caused by the enzyme-substrate imbalance between ADAMTS13 and UL-VWFM.

Substantial increase of plasma VWF levels according to progression of liver diseases has been reported previously[25,26]. This is probably due to endothelial damage of the hepatic sinusoid caused by endotoxin and cytokines[25-28]. Hepatic cell necrosis and subsequent liver regeneration, and/or high shear stress due to portal hypertension in cirrhotic liver may play major roles in up-regulating VWF in hepatic sinusoidal endothelium. The mechanism responsible for the decrease of ADAMTS13 activity in advanced cirrhotic patients may include enhanced consumption due to a degradation of a large quantities of VWF[21], inflammatory cytokines[29,30], and/or ADAMTS13 plasma inhibitor[13,31]. These findings in patients with liver cirrhosis suggest that imbalance between ADAMTS13 and UL-VWFM can induce liver dysfunction due to microcirculatory disturbance.

It is well-known that ticlopidine, which is one of the most popular antiplatelet agents, can be a cause of severe deficiency of ADAMTS13 activity, the condition known as TTP[32]. The drug may induce inhibitor of ADAMTS13. Because the patients with cirrhosis are prone to deficiency of ADAMTS13 activity, the use of ticlopidine is better to be avoided as possible.

ASSAY SYSTEM OF ADAMTS13 AND UL-VWFM

The article contains some original data in the part of liver transplantation. ADAMTS13 and UL-VWFM were measured with the methods described below. Written informed consent was obtained in all patients in whom the blood samples were used for the assay.

Traditionally, the activity of plasma ADAMTS13 was measured by the multimer method using full length VWF as a substrate according to the method reported by Furlan *et al*[12], although we made slight modification to this method as described in our previous study[15]. The method required at least 3 d to assay the activity of ADAMTS13. Our newly developed method is enzyme-linked immunosorbent assay (ELISA) using a specific murine monoclonal antibody to Tyr1605 residue of VWF-A2 domain which is generated by ADAMTS13 cleavage. Rrecombinant GST-VWF73-His polypeptide is used as a substrate[33]. One of the advantages of this method is that the assay time is significantly shortened. This new method is more sensitive and more rapid than the traditional multimer method. This ELISA kit is available on commercial base now. Plasma UL-VWFM can be analyzed by vertical agarose gel electrophoresis as described[15].

IMPACT OF ADAMTS13 IN PATIENTS WITH LIVER TRANSPLANTATION

We have revealed significant decrease of ADAMTS13 activity in very sick children with advanced cirrhotic biliary atresia (BA) and full recovery of the activity after living-related liver transplantation (LRLT). This finding strongly suggested that the liver is the major source of ADAMTS13. Briefly, 8 pediatric patients with BA received LRLT from adult live donors, indicating that almost a normal size of liver was transplanted into the recipients (Table 1). Before LRLT, plasma ADMTS13 activity showed a significant decrease in 7 out of 8 patients, and the value for two patients (cases 1 and 8) was extremely low at 13% and 6% of the control, respectively. One to three months after successful LRLT, six patients showed an increase in ADAMTS13 activity. It is noteworthy that decreased ADAMTS13 activity restored by liver transplantation in the majority of patients (Figure 1). Table 1 and Figure 1 are original data of our research team. With regard to the increase in ADAMTS13 activity after LRLT, there were two possible explanations. One is simply that the liver is the major organ to synthesize ADAMTS13, like blood coagulation factors Ⅷ and Ⅸ previously shown in hemophiliacs who received liver transplantation[34-36]. Another explanation might be that liver dysfunction produces a harmful substance which may affect the systemic production or activity of ADAMTS13. However, the presence of a substance interfering with the enzyme activity is plausible, because no inhibitor of ADAMTS13 was detected in sick BA patients. While the site of ADAMTS13 synthesis still remained to be elucidated at the time of study about pediatric BA patients, the results strongly suggested that the liver is the critical organ in the synthesis of ADAMTS13. Following this finding, our research group performed *in situ* hybridization analyses of the liver, which revealed that stellate cells (Ito cells) of sinusoid of the liver produced ADAMTS13[7].

Subsequently, we experienced one noticeable adult patient who developed severe thrombocytopenia soon after LRLT because of advanced liver failure due to Budd-Chiari syndrome (Figure 2). The analysis of the thrombocytopenic mechanism in this patient gave a

Table 1 Profiles of patients who received living-related liver transplantation

	Recipient						Donor				
Case	Age	Sex	Underlying disease	ABO-Rh (D) blood group	SV (g)	Presence of schistocytes before LRLT	Relation to the recipient	Age	ABO-Rh(D) blood group	GV (g)	GV/SV ratio
1	9 mo	M	BA	A+	274	+	Father	35 yr	A+	290	1.06
2	1 yr 4 mo	F	BA	A+	245	+	Mother	38 yr	A+	314	1.28
3	12 yr	F	BA	A+	891	-	Mother	44 yr	A+	392	0.44
4	11 mo	F	BA	A+	259	+	Mother	35 yr	A+	306	1.18
5	8 mo	F	BA	O+	185	+	Father	36 yr	O+	263	1.42
6	1 yr	F	BA	A+	262	+	Mother	41 yr	O+	215	0.82
7	11 mo	F	BA	AB+	280	-	Mother	35 yr	A+	266	0.95
8	7 mo	M	BA	A+	226	-	Mother	30 yr	A+	228	1.01

LRLT: Living-related liver transplantation; SV: Standard liver volume; GV: Graft liver volume; BA: Biliary atresia; M: Male; F: Female.

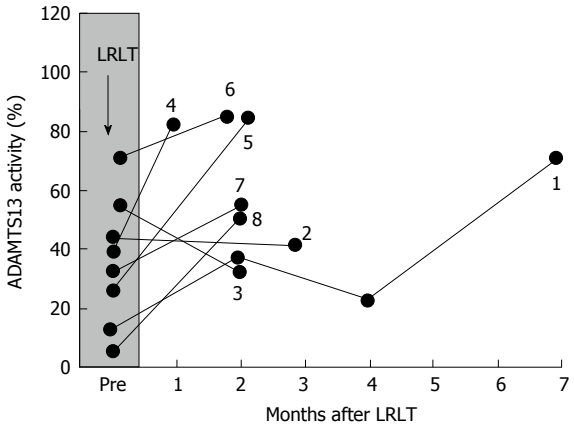

Figure 1 Plasma a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 activity before and after living-related liver transplantation. Predominantly decreased ADAMTS13 activity could be fully restored after living-related liver transplantation in 6 out of 8 sick children with advanced cirrhotic biliary atresia (cases 1, 4, 5, 6, 7 and 8). Pre: Before transplantation; LRLT: Living-related liver transplantation; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

paradigm shift, which closely linked the axis of VWF-ADAMTS13 reaction to liver transplantation. Briefly, during his uneventful clinical course in the early stage after liver transplantation, the platelet count decreased gradually from 83000/μL to 62000/μL until postoperative day 5, and decreased further to 25000/μL until day 7 (Figure 2, left). While the patient developed graft liver dysfunction due to acute rejection around day 7, no possible causes of thrombocytopenia were found from clinical findings and usual laboratory tests. To make a decision of platelet transfusion to this patient, plasma ADMTS13 activity was assessed. This was because it has been said that prophylactic platelet transfusion is better avoided to TMA-patients, who had no manifestations of overt bleeding[37]. The result of assay showed a remarkable decrease of ADAMTS13 activity to 3% from 108% before surgery. Presence of UL-VWFM in patient plasma was also identified apparently on day 1 and day 7 using SDS-agarose gel electrophoretic analysis (Figure 2, right panel). Transfusion of a large amount of fresh frozen plasma (FFP) as a treatment for TMA together with a high-dose steroid pulse therapy as treatment for acute rejection resulted in recovery of thrombocytopenia and liver function, and substantial increase of ADMTS13. Thereafter, the ADAMTS13 activity finally recovered to 50%, corresponding to the lower limit of the normal range, until day 98. The UL-VWFM tended to diminish on day 15, but again became prominent on day 22 during the second episode of acute rejection, and became undetectable until day 45. Profound decrease of plasma ADAMTS13 is a specific finding of TMA which is known as a sporadic serious complication after solid organ transplantation with an estimated frequency of 0.5%-3.0%[38-41]. However, the patient never showed any apparent clinical features including renal dysfunction, neuro-psychological symptoms or hemolytic anemia, as typically seen in TMA[41]. Because the patient certainly had a remarkably increased plasma level of VWF, together with the presence of UL-VWFM, we have supposed that platelet transfusion might generate microcirculatory disturbance due to the enhanced micro-thrombi formation, initially localized to the transplant liver, but later could be expanded systematically. This idea came from the findings, in which liver transplantation often accompanies with the endothelial cell damage due to ischemia-reperfusion injury and acute rejection. In fact, the injured hepatic vascular endothelial cells may release a large quantity of VWF/UL-VWFM[42,43], which may induce a consumption of ADAMTS13 in plasma. Then we supposed new hypothesis that the initiation of pathological mechanism may occurs locally at the site of the transplanted liver.

Based on these findings, we have started analysis of ADAMTS13 activity and UL-VWFM in patients after liver transplant recipients. The results revealed that significant decrease of ADAMTS13 and up-regulation of UL-VWFM were commonly and frequently observed after liver transplantation without findings of usual systemic TMA or TTP[15]. These changes in ADAMTS13 activity and UL-VWFM were relevant to posttransplant liver dysfunction, including ischemia-reperfusion injury and acute rejection (Figure 3). Many of patients with decreased ADAMTS13 activity showed concurrent thrombocytopenia. The clinical manifestation was analogous with TMA, especially to TTP. However, different from TTP, the deterioration was restricted to the transplanted liver, without development

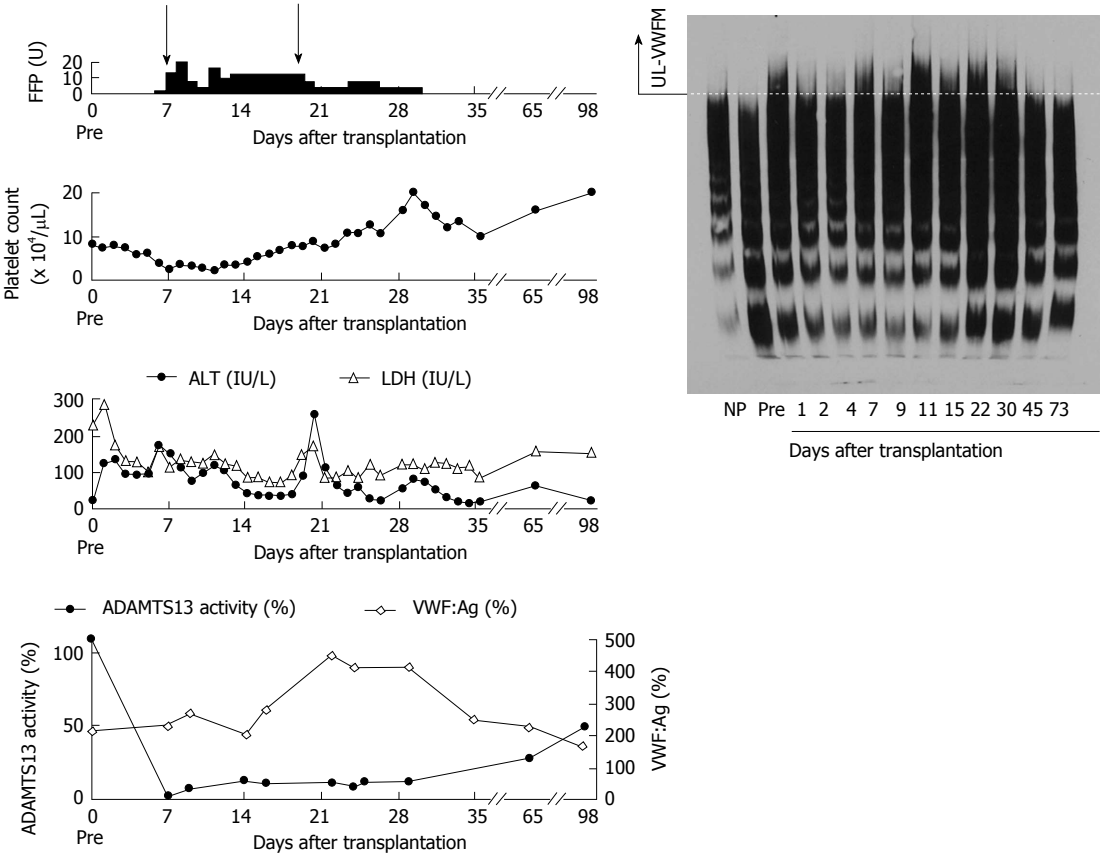

Figure 2 Clinical course of 27-year-old male with Budd-Chiari syndrome after liver transplantation. Serum ALT level mildly increased on days 1 and 2 because of ischemia-reperfusion injury, decreased thereafter, but rapidly increased again on day 7 due to acute rejection (left panel). The platelet count decreased gradually and reached a nadir on day 7, when ADAMTS13 activity decreased markedly to less than 3% from 108% before surgery. After the administration of fresh frozen plasma and bolus injection of methylprednisolone (arrow), ALT level decreased and the platelet count gradually increased. The ADAMTS13 activity increased to 22% on day 14. After the first episode of acute rejection, VWF:Ag increased further and reached 368% on day 21, when ALT again increased due to a second episode of acute rejection. Bolus injection of methylprednisolone (arrow) led to a rapid decrease of ALT and a gradual increase in the platelet count. VWF:Ag decreased gradually, and ADAMTS13 activity finally recovered to 50% until day 98. Plasma UL-VWFM was detectable on day 1 at the time of ischemia-reperfusion injury, thereafter diminishing gradually during days 2 to 4, and again becoming evident on day 7 when acute rejection developed (right panel). The UL-VWFM disappeared transiently on day 9, but reappeared on day 11, coinciding with a mild increase of transaminase. UL-VWFM tended to diminish on day 15, but again became prominent on day 22 during the second episode of acute rejection. Pre: Before transplantation; NP: Normal plasma control; ALT: Alanine transaminase; LDH: Lactate dehydrogenase; FFP: Fresh frozen plasma; VWF: von Willebrand factor; UL-VWFM: Unusually large VWF multimers; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

of renal dysfunction or neurological disorders which were characteristic to usual TTP. Then we have advocated "local TTP like mechanism" for the first time as a pathogenesis of imbalance between plasma ADAMTS13 and UL-VWFM in liver transplant recipients[15].

It was reported that increased numbers of activated platelets and VWF expression were indicated in the hepatic sinusoidal endothelial cells of the re-perfused or cold-preserved liver[42,43]. VWF expression was increased significantly in the grafted liver with acute rejection due to allogenic immune response[43]. After up-regulation of VWF, formation of UL-VWFM on the vascular endothelial cells may induce platelet thrombi in the hepatic sinusoid. This may be the mechanism of microcirculatory disturbance due to imbalance between ADAMTS13 and UL-VWFM in liver transplant recipients. This hypothesis explains clearly why organ dysfunction restricts to the grafted liver in liver transplant recipients with low ADAMTS13 activity, distinct from systemic organ disorders in patients with "classical TTP".

Recent report by Kobayashi *et al*[44] confirmed our findings by showing cross correlation between decrease of ADAMTS13 activity and thrombocytopenia in 81 liver transplant recipients. They also showed increased levels of VWF and up-regulated UL-VWFM. More recently, Hori *et al*[45] reported poor outcomes in patients who developed TMA like disorder after liver transplantation. Most of these patients showed marked decrease of ADAMTS13 activity, while they did not include ADAMTS13 activity in the diagnostic criteria of the disorder. Cross correlation between low ADAMTS13 activity and poor outcomes of the patients was shown in their study.

Following our study[15], another group also reported that a reduction of ADAMTS13 activity after liver transplantation[46]. They insisted that reduction of ADAMTS13 correlated to thrombotic complication by showing a patient with hepatic artery thrombosis. However, different from our findings, they failed to detect UL-VWFM in any

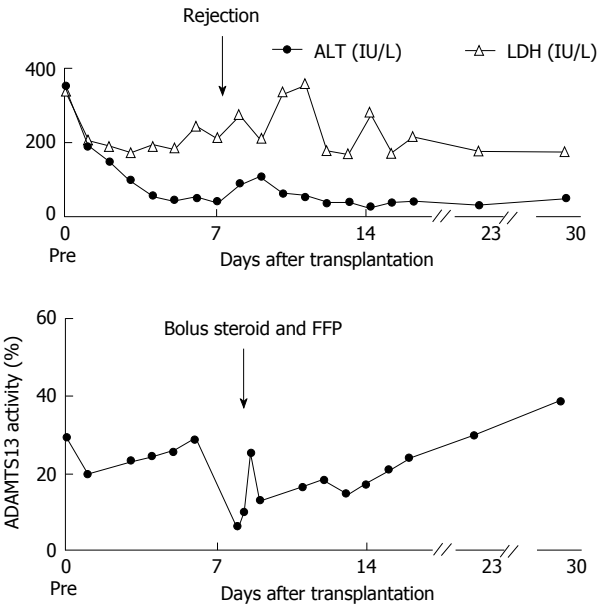

Figure 3 A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 activity before and after treatment for acute rejection in a liver transplant recipient. Decreased ADAMTS13 activity during acute rejection recovered after treatment for rejection. Pre: Before transplantation; FFP: Fresh frozen plasma; ALT: Alanine transaminase; LDH: Lactate dehydrogenase; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

patients with decreased ADAMTS13 activity. The authors speculated that increased plasmin or other proteolytic enzymes cleaved UL-VWFM demonstrating increased plasma levels of tissue plasminogen activator in these patients[46,47]. However, if plasmin cleaves UL-VWFM effectively, thrombotic complications could be prevented. Actually, it has been accepted commonly that UL-VWFM is detected in patients with TTP[20,48]. This may suggest that the deficiency of ADAMTS13 can induce formation of UL-VWFM independently of plasmin and other proteolytic pathways. In addition, hepatic artery thrombosis is a complication crossly linked technical and anatomical implications at the arterial anastomotic site. Basically, ADAMTS13 relates to the formation of platelet thrombi at the "micro" vasculature. Thrombosis of peripheral arterial system as a consequence of microcirculatory disturbance of the graft liver and surgical hepatic arterial thrombosis at the anastomotic site should be distinguished strictly to elucidate the real correlation between ADAMTS13 and the development of thrombotic complications after liver transplantation.

Our experience in liver transplant recipients strongly emphasize the importance of monitoring plasma ADAMTS13 activity not only in diagnosing adverse events including ischemic injury and acute rejection, but also in the treatment of thrombocytopenia after liver transplantation. It should be stressed that platelet transfusion under the decreased activity of ADAMTS13 in liver transplant recipients may deteriorate graft microcirculatory disturbance due to further formation of platelet aggregates in the liver *via* the "local TTP" mechanism[15]. Liver dysfunction with marked decrease of ADAMTS13 activity should be treated with a large amount of FFP or plasma exchange as indicated for TTP, even when the patient shows no clinical signs of usual TTP.

KINETICS OF ADAMTS13 AFTER HEPATECTOMY FOR LIVER TUMORS

Hepatectomy is mainly indicated for treatment of liver tumors. Because hepatic functional mass is reduced after hepatectomy, thrombo-hemostatsis related agents produced in the liver may decrease at least in the early phase before regeneration of the residual liver. Because ADAMTS13 is produced mainly by the hepatic stellate cells[7], production of ADAMTS13 may decrease after hepatetctomy. In addition, ischemia-reperfusion injury of the liver is an inevitable event in liver surgery due to manipulation of the liver and/or Pringle's maneuver which occludes hepatic blood inflow transiently to decrease bleeding during hepatic transection[49]. Therefore, hepatectomy may induce UL-VWFM in the hepatic sinusoid and result in microcirculatory disturbance *via* the "local TTP like mechanism" as mentioned in the liver transplantation section. Liver failure is a most serious complication after liver surgery. Microcirculatory disturbance may further deteriorate liver dysfunction after hepatectomy.

We reported that plasma ADAMTS13 decreased significantly after hepatectomy[16]. The activity of ADMTS13 showed marked and rapid drop from 67% ± 30.6% before surgery to 48% ± 24.6% (mean ± standard deviation) on day 1 after hepatectomy (n = 70, $P < 0.0001$)[16]. The decrease of ADAMTS13 activity was more profound in patients with major hepatectomy in comparison to those with minor hepatectomy[16]. Multivariate analysis revealed that patients with Pringle's maneuver for longer than 60 min induced most marked decrease of ADAMTS13 activity compared to those with shorter Pringle's maneuver and those without Pringle's maneuver (Figure 4). The severity of ADAMTS13 reduction was significantly correlated with the amount of resected liver mass and the severity of ischemic injury of the liver. ADAMTS13 activity on day 1 strongly correlated with the postoperative maximal levels of total bilirubin as an indicator of postoperative liver dysfunction[16]. These results suggested crucial roles of ADAMTS13 in liver dysfunction and liver failure after hepatectomy.

Figure 5 shows kinetics of ADAMTS13 and UL-VWFM in a patient who underwent major hepatectomy with long Pringle's maneuver. Interestingly, ADAMTS13 activity did not decrease during ischemia by Pringle's maneuver, and decreased very significantly after re-perfusion until the next day. UL-VWFM did not appear during ischemia, and significantly up-regulated after surgery. Induction of UL-VWFM and decrease of ADAMTS13 may develop in the reperfusion phase after ischemic events.

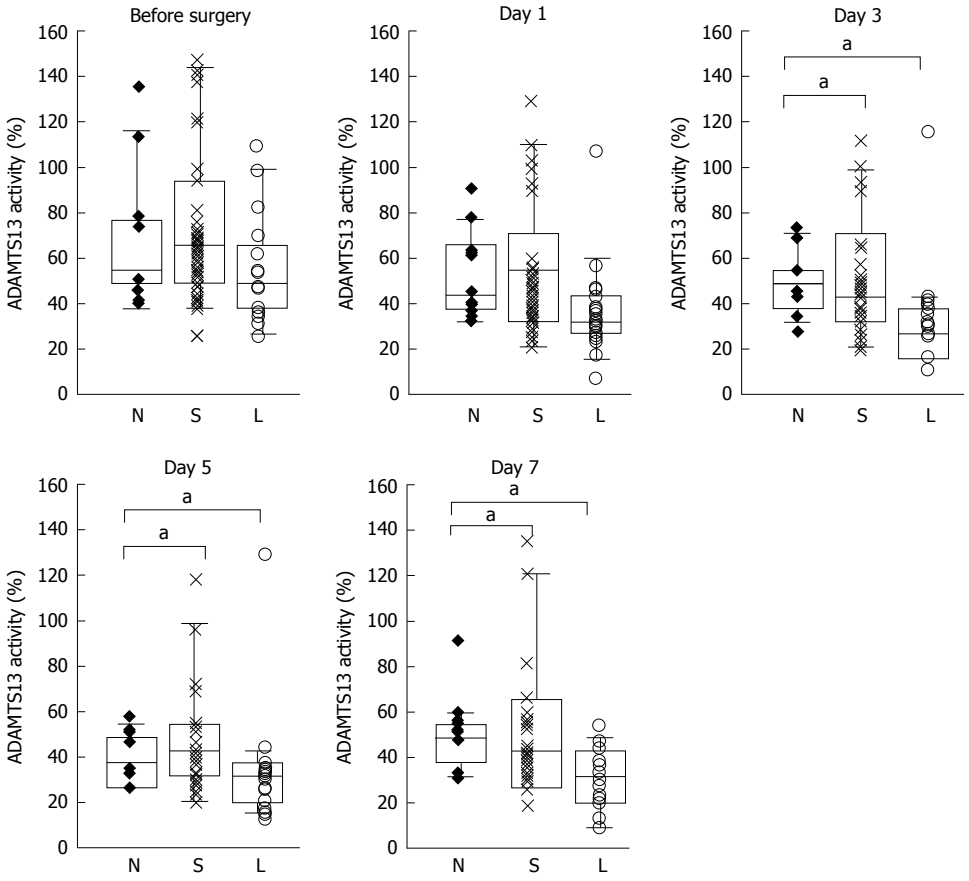

Figure 4 A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 activity according to blood occlusion time by Pringle's maneuver. Pringle's maneuver for long time (over 60 min: L) induced significantly profound decrease of ADAMTS13 activity, comparing to short (15-45 min: S) or no blood occlusion (N). The box shows the 25th-75th percentile, the bar indicates the median value, and the whiskers indicates the 5th-95th percentile. $^{a}P < 0.05$ by ANOVA. ANOVA: Analysis of variance; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

Marked decrease of platelet count with unexplainable origin is often observed during the first postoperative week after hepatectomy. In our study, significant correlation was observed between ADAMTS13 activity and decrease of platelet count[16]. Reduction of ADAMTS13 synthesis by decreased hepatic functional mass after hepatectomy may be a cause of decreased plasma ADAMTS13 activity. Another possible mechanism of marked decrease of ADAMTS13 may be high share stress after major hepatectomy. Major hepatectomy increases relative amount of portal inflow of residual liver. Because UL-VWFM is stretched by high share stress, a large amount of ADAMTS 13 may be consumed (Figure 6). Decrease of ADAMTS13 may be a possible indicator of postoperative liver dysfunction. From our findings, 40% may be the safe line of ADAMTS13 activity after hepatectomy[16]. Replacement therapy for decreased ADAMTS13 may prevent postoperative liver dysfunction.

Recent study also reported decrease of ADAMTS13 after major hepatectomy[50]. The study showed correlation between increased VWF to ADAMTS13 ratio and thrombotic complication after major hepatectomy. Melloul *et al*[51] refered to the possible role of ADAMTS13 activity in development of pulmonary embolism after hepatectomy. Correlation between overt embolism and decreased activity of ADAMTS after hepatectomy is needs to be further elucidated.

THROMBOCYTOPENIA AND TRANSFUSION OF PLATELET CONCENTRATES

Various degrees of thrombocytopenia are commonly observed in cirrhotic patients and in those who received surgical interventions, such as liver transplantation and hepatectomy[52,53]. Before discovery of ADAMTS13, transfusion of platelet concentrates to these patients has been simply and routinely performed to prevent hemorrhagic events. But after discovery of ADAMTS13, the investigators have become cautious to platelet transfusions to such patients. This is because our groups of investigators have shown that a significant decrease of plasma ADMTS13 activity and vice versa a remarkably high plasma concentration of VWF, containing UL-VWFM, are both frequently observed in patients with various liver diseases including surgical interventions[15,16,20]. These circumstances generate an extremely high plasma ratio of VWF or UL-VWFM to ADAMTS13, that lead to an unstable condition forming platelet thrombi by UL-VWFM

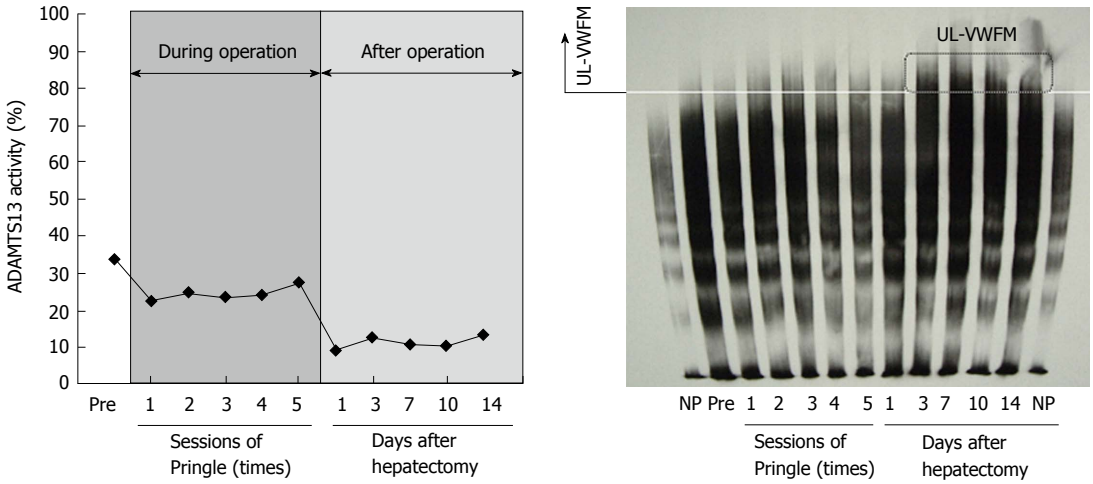

Figure 5 Perioperative changes of a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 activity and unusually large von Willebrand factor multimers in a patient with large hepatectomy. The patient underwent long Pringles's maneuver (75 min). While ADAMTS13 did not decrease during hepatectomy even with long ischemic time by hepatic inflow occlusion, the activity markedly decreased until the next day. Consistently, UL-VWFM did not appeared during hepatectomy, and significantly up-regulated from day 3. Pre: Before transplantation; NP: Normal plasma controls; UL-VWFM: Unusually large von Willebrand factor multimers; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

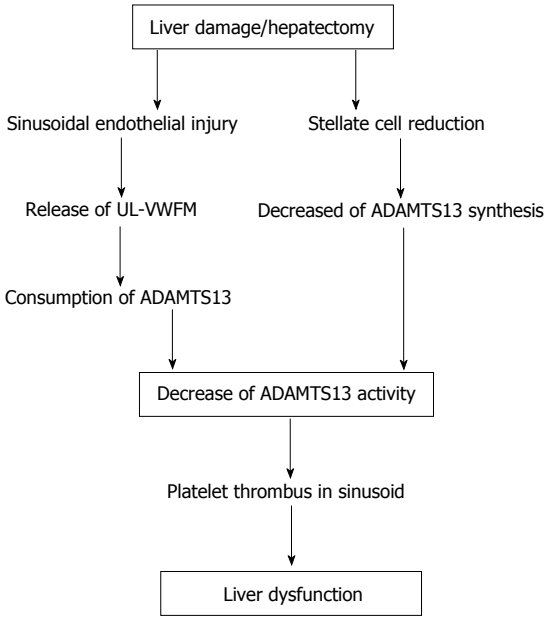

Figure 6 Hypothesis about mechanism of liver dysfunction *via* the local thrombotic thrombocytopenic purpura like mechanism. UL-VWFM: Unusually large von Willebrand factor multimers; ADAMTS13: A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13.

under high shear stress generated in microvasculatures, and may induce multiple organ failure resembling to TTP. Actually, porto-pulmonary hypertension exacerbated by platelet transfusion in a patient with ADAMTS13 deficiency due to platelet aggregation in microcirculation of the liver and lung, and the pulmonary arterial pressure fell after replacement of plasma ADAMTS13 by infusion of FFP[54]. Thus, our opinion is that the measurement of plasma ADAMTS13 activity is pre-requisite during the clinical course in these patients, and the prophylactic platelet infusion is better to be avoided or rather contraindicated. However, we also must emphasize that platelet transfusions should be performed if overt bleeding once developed, supplying ADAMTS13 by infusion of FFP simultaneously.

CONCLUSION

ADAMTS13/UL-VWFM paradigm, which we advocated, is a new concept in the field of liver disease and surgery. The partnership between hematology and hepatology not only suggests a novel mechanism for thrombocytopenia, but also provides a useful diagnostic tool for the treatment of thrombocytopenia and liver dysfunction in patients with various liver diseases. Introduction of ADAMTS13 activity assay system as a routine clinical laboratory tests may help to prevent inadequate platelet transfusion. The efficacy of preemptive supplementation of ADAMTS13 activity by administrating FFP as a source of ADAMTS13 after liver surgery should be investigated in view of ADAMTS13/UL-VWF dynamics. We are particularly interested in developing recombinant ADAMTS13 preparations, which provides a new therapy for patients with hematologic and various liver diseases.

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ORIGINAL ARTICLE

Observational Study

Impact of geography on organ allocation: Beyond the distance to the transplantation center

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Ethics approval statement: The study was approved by the institutional review board. Given that the project is based on data from the United Network of Organ Sharing (UNOS) which is already de-identified, it was not deemed a human research project. A copy of the IRB statement is provided as an attachment.

Informed consent statement: The study is based on the UNOS registry, an additional informed consent from the patients does not apply.

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

Data sharing statement: The statistical code and dataset are available from the corresponding author at rony.ghaoui@bhs.org. There is no technical appendix. Consent was not obtained but the presented data are anonymized and were obtained from the UNOS hence there is no risk of identification. A copy of the signed statement is provided as an attachment.

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Abstract

AIM: To illustrate the application and utility of Geographic Information System (GIS) in exploring patterns of liver transplantation. Specifically, we aim to describe the geographic distribution of transplant registrations and identify disparities in access to liver transplantation across United Network of Organ Sharing (UNOS) region 1.

METHODS: Based on UNOS data, the number of listed transplant candidates by ZIP code from 2003 to 2012 for Region 1 was obtained. Choropleth (color-coded) maps were used to visualize the geographic distribution of transplant registrations across the region. Spatial interaction analysis was used to analyze the geographic

pattern of total transplant registrations by ZIP code. Factors tested included ZIP code log population and log distance from each ZIP code to the nearest transplant center; ZIP code population density; distance from the nearest city over 50000; and dummy variables for state residence and location in the southern portion of the region.

RESULTS: Visualization of transplant registrations revealed geographic disparities in organ allocation across Region 1. The total number of registrations was highest in the southern portion of the region. Spatial interaction analysis, after adjusting for the size of the underlying population, revealed statistically significant clustering of high and low rates in several geographic areas could not be predicted based solely on distance to the transplant center or density of population.

CONCLUSION: GIS represents a new method to evaluate the access to liver transplantation within one region and can be used to identify the presence of disparities and reasons for their existence in order to alleviate them.

Key words: Health geographic's; Healthcare disparities; Outcome research

Core tip: Geographic Information System (GIS) studies the impact of geography on many problems through statistical modeling and analysis. It has been used to guide decisions in business, government, environment, but has yet to be adopted in healthcare. Based on the United Network of Organ Sharing database from 2003 to 2012 in one region, GIS revealed clustering of high and low rates of listing for liver transplantation in several geographic areas that could not have otherwise been predicted. This method can be adopted in different parts of the world and contribute to better allocation of resources to decrease the disparities in access to liver transplantation.

Ghaoui R, Garb J, Gordon F, Pomfret E. Impact of geography on organ allocation: Beyond the distance to the transplantation center. *World J Hepatol* 2015; 7(13): 1782-1787 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i13/1782.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i13.1782

INTRODUCTION

Liver transplantation remains the only truly therapeutic intervention for patients with end stage liver disease and decompensated cirrhosis. Unfortunately, the access to organ transplantation from a cadaveric or living donor is limited by a shortage of donors. This has become especially problematic given the expected surge of patients in need of a transplant due to the combination of three epidemics: (1) Hepatitis C that may lead to a quadrupling of the number of patients with decompensated cirrhosis in 10 years[1]; (2) the obesity epidemic leading to cirrhosis through non-alcoholic fatty liver disease[2]; and (3) hepatocellular carcinoma[3].

This alarming shortage of organs is likely going to exacerbate the established healthcare disparities including the ones stemming from Geography. With regard to access to liver transplantation, 7 of 11 United States regions differed significantly from the national average[4]. This has clear implications on the management of patients with hepatocellular carcinoma; a United Network for Organ Sharing (UNOS) database has showed that regions with longer waiting times used more loco-regional palliative therapy[5]. This occurs despite tumor allocation points that aim at giving an advantage to liver cancer candidates, especially with long waiting time on the transplant lists[6]. In the United States and based on a study from Region 4, it was suggested that the distance from the transplant center should be included to improve the estimate of the mortality risk for patients on the waiting list[7].

A Geographic Information System (GIS) represents a systematic way to study the impact of geography on this problem. GIS can synthesize data from several different sources, visualize trends in maps that would not otherwise be apparent, and reveal significant spatial associations through statistical modeling and analysis. GIS has long been used to guide decision-making process in disparate fields such as business, government, environment and conservation, the military and epidemiology. The impact of GIS in strategic decisions in healthcare has not been widely adopted.

Accordingly, the aim of our study was to demonstrate the utility of GIS with organ transplantation data to assess geographic disparities in burden of care and access to liver transplantation across UNOS region 1 and to examine the stability of these disparities over time. We also wanted to determine if geography should be considered as a factor in building satellite clinics to evaluate patients for liver transplantation.

MATERIALS AND METHODS

The total number of transplant registrations (listed candidates) for UNOS Region 1 by ZIP code from 2003 to 2012, was obtained from UNOS. This represents the total transplant burden of care on local transplant facilities or need. ZIP code geographic data was originally obtained as geographic coordinates and was projected to Lambert Conformal for visualization and analysis. The number of transplant registrations by ZIP code were mapped with ArcGIS software[8] using a choropleth (color-coded) map, where number of registrations is represented by the intensity of color. Spatial interaction analysis based on the gravity model was used to analyze the geographic pattern of total transplant registrations by ZIP code. Transplant registrations were analyzed for all years combined to stabilize numbers. The gravity

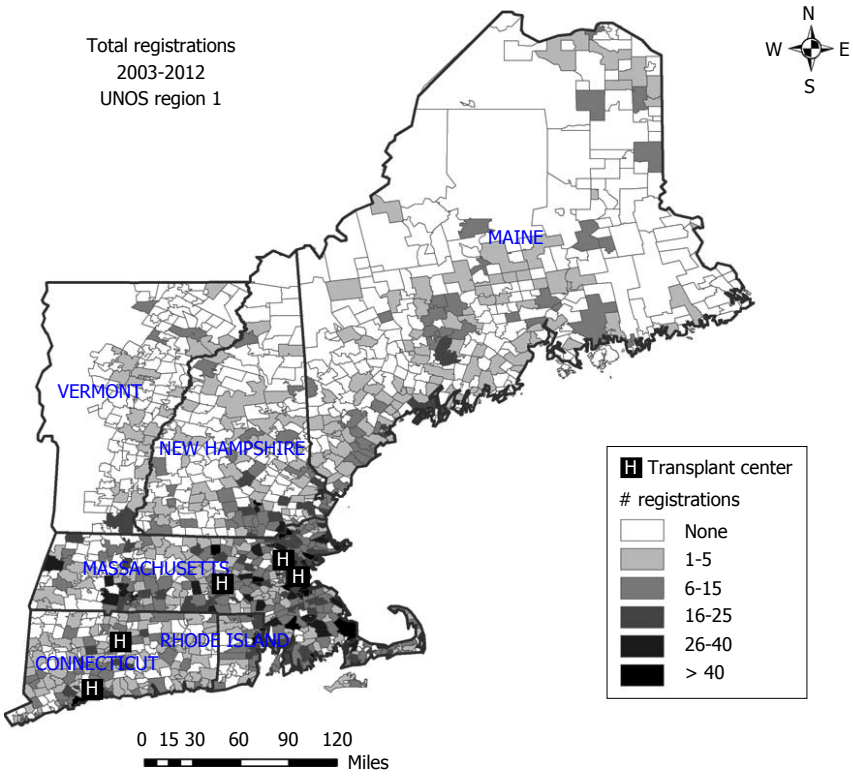

Figure 1 United Network of Organ Sharing Region 1 ZIP code transplant registrations 2003-2012. UNOS: United Network of Organ Sharing.

model is used to model flow (in this case, number of transplant registrations) from one or more origins (in this case, ZIP code) to one or more destinations (in this case, transplant centers). The model assumes that flow is determined by the population of the origin and distance from the origin to the destination[9]. Poisson regression was used to derive the gravity model using STATA software[10]. Factors tested included ZIP code log population and log distance from each ZIP code to the nearest transplant center (both specified by the gravity model); ZIP code population density; distance from the nearest city over 50000; and dummy variables for state residence and location in the southern portion of the region (consisting of Massachusetts, Connecticut or Rhode Island) which houses all the region's transplant centers.

To adjust for the size of the underlying population, ZIP code registration rates were calculated as the number of candidates registered in a ZIP code divided by the total ZIP code population for each time period. Census 2000 population counts were used to calculated 2003-2005 rates, and Census 2010 counts were used for 2006-2012 rates.

If services were equally accessible throughout the region, registration rates should not vary geographically. To identify regional disparities in accessibility, we looked for clusters of excessively high or low rates relative to the rest of the region by year using the Spatial Scan Statistic[11]. The geographic locations of high and low clusters identified in this manner were then displayed using choropleth mapping. The statistical review of the study was performed by a biomedical statistician.

RESULTS

Table 1 gives the total number of registrations (burden of care) and registration rates (accessibility) by state for the total 10-year period. States are arranged in ascending order according to registration rate. There is great disparity in rates according to state. Massachusetts had the highest rate, almost three times that of Connecticut, which had the lowest rate. In Region 1, liver transplant programs include 5 in Massachusetts and 2 in Connecticut.

To visualize geographic variability in burden of care, Figure 1 shows the total number of transplant registrations by ZIP code for 2003-2012. ZIP codes with higher numbers of registrations are represented in darker shades of gray or black. Those with no registrations in the 10-year period are represented in white. As can be seen, there is wide variability in total registration numbers across the region, generated in part by variability of population size. A large number of ZIP codes had no transplant registrations. Visually, it is apparent that the number of registrations was highest in the southern portion of the region, in Massachusetts, Connecticut and Rhode Island.

Factors in burden of care were examined with Poisson regression on registration totals. As predicated by the gravity model, registrations showed a strong, significant association with between population size and total number of registrations indicated by the *Z*-value (25.03)

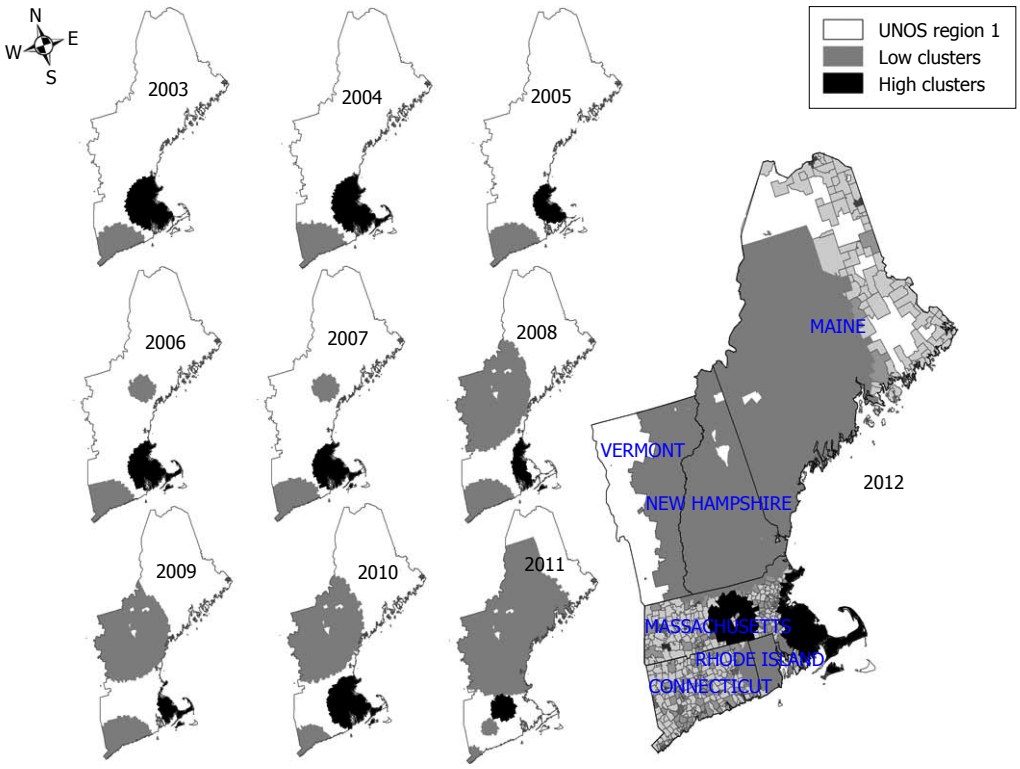

Figure 2 Clustering of ZIP code listing rates over time. UNOS: United Network of Organ Sharing.

Table 1 Total registrations by state

State	Total registrations	Rate per 100000 population
CT	1024	29.0
VT[1]	104	41.6
ME	637	48.4
NH	652	50.0
RI	655	62.8
MA	5241	80.4

[1]Partial-only a portion of the state is included in United Network of Organ Sharing Region 1. CT: Connecticut; VT: Vermont; ME: Maine; NH: New Hampshire; RI: Rhode Island; MA: Massachusetts.

and *P*-value (< 0.001). Distance from a transplant center was not significant (P = 0.1), implying that the gravity model does not completely specify registration patterns. The model did, however, account for 31% of the variability in registration numbers (R^2 = 0.31). Other factors examined that were not significant were population density, location in the south of the region and distance to the nearest city > 50000 population.

To visualize disparities in accessibility of transplant services, Figure 2 shows the location of high and low clusters of registration rates per 100000 population in the region each year from 2003-2012. The 2012 map is shown at a smaller scale for more detail. A cluster is defined as a group of ZIP codes with significantly higher or lower registration rates than the rest of the region. Low clusters are represented as gray areas, and high clusters as black areas. It is clear from the map that there was very little variation over time in the location of high clusters, which centered on the Southeast corner of the region in Rhode Island, Eastern Massachusetts and Southeast New Hampshire. There has been somewhat more variation in the location of low clusters over time. However, these clusters were generally confined to Connecticut and to the central sections of the region, in New Hampshire, Vermont and Maine. Table 2 shows the details of significant clusters in 2012. As can be seen, the most significant cluster, which included 63 ZIP codes and a relatively small population, had a registration rate 3 times the prevailing rate in the region. The other clusters covered a larger area and represented a larger population.

DISCUSSION

With its relatively small geographical extent and the availability of numerous health systems of global reputation, one would expect that the impact of geography on organ allocation in Region 1 would be limited. The analysis of the UNOS data with a GIS brings a new perspective. The maps generated revealed clusters of excessively high listing rates for ZIP codes in the Boston area that remained stable over a decade with an increasing disparity and worsening access in the northern part of the region. More intriguing is the revelation of a new cluster of excessively low registration rates covering a good portion of Rhode Island despite its close driving distance to several transplant centers in 2012. This relates to the finding in multivariate analysis that distance to a transplant center as a predictor of listing rates

Table 2 Details of significant clusters in 2012

Cluster	Cluster type	Listed	Expected	Relative risk	Significance	ZIP codes	Population
1	High	114	39	3.3	<< 0.0001	63	683114
2	High	198	144	1.5	0.039	165	2554406
3	Low	107	171	0.57	0.00025	710	3027545
4	Low	34	67	0.48	0.055	89	1191886

implies that adding transplant centers in areas with low listing rates would not solve the problem. It seems more likely that local characteristics explain this phenomenon including, but not limited to, variable access to healthcare and subspecialists, educational and cultural beliefs related to transplantation. However this is difficult to prove in the absence of reliable databases.

Another geographical area worth investigating further is Connecticut with a progressive transition to a homogenous registration pattern over the past decade which correlates with the reopening of a transplant center. A practical conclusion from our study might be to consider establishing satellite clinics in the low-cluster areas with a connection to a main transplant centers following a model like the ECHO project. This initiative in New Mexico demonstrated that primary care providers anywhere can be trained *via* videoconferencing technology to manage complex chronic conditions formerly outside their expertise, thus expanding their ability to treat very sick patients and showing equal outcomes to academic settings[12]. In a setting of transplantation these satellite clinics could allow a better understanding of the local barriers preventing optimal access to listing for liver transplantation not to mention optimizing the care of patients with decompensated cirrhosis.

Our study certainly has limitations. We chose to study the listing rates for liver transplantation and not the actual organ recipients given the smaller number of the latter group which would have left blank vast portions of the map. Our analysis assumes a similar effectiveness of the organ procurement organizations across the region as well as a homogeneous distribution of the burden of chronic liver disease. We could not obtain data on individual level patient characteristics and our statistics are based on aggregate numbers only. ZIP codes have limited utility as unit of analysis as they cover large demographically heterogeneous geographic areas. Also the population counts are estimates, as the census data is not collected at the ZIP code level. Despite these limitations our results reveal striking trends and generate hypotheses for further studies.

There is a clear perception, and often direct knowledge among patients and providers alike, that geography impacts organ allocation. This regional disparity, which was also seen outside of the United States[13], is often assumed to be the result of the distance between the patient's home and the transplant center and its policy. This contention is not supported by our analysis, suggesting a more complex situation. As reported previously, significant variations in access to liver transplantation for ethnic minorities continue to be seen across geographic lines[14]. Furthermore in Canada, rural residence of a candidate was not associated with inferior survival while awaiting liver transplantation[15]. Our analysis using GIS explores the distribution of listing for liver transplantation from a new perspective, raising several points that once further clarified could lead to a better understanding of the impact of geography and how to mitigate it: What are the policies or changes in Connecticut that have allowed a more homogenous outcome after a decade? Why did large portions of Rhode Island lose their better access in 2012? How do we contain the worsening disparities seen in the northern part of the region? These questions should find their answers with a closer collaboration and communication among stakeholders in the region.

The analysis of the UNOS data of registrations for liver transplantations using GIS has challenged several assumptions including the absence of disparity within a small geographical area or the stability of this disparity over time. It has also revealed that the impact of geography goes beyond distance to the transplant center and needs to be further evaluated. Future studies should explore and analyze the UNOS data in other regions and for other types of organ transplantation. Only then would we be able to identify low-cluster areas for organ transplantation across the nation. This is crucial at a time when healthcare is redefined by value and quality and redistricting for organ allocation might be on the way[16].

COMMENTS

Background

The prevalence of chronic liver disease leading to cirrhosis or end stage liver disease continues to increase worldwide given the epidemics of hepatitis C, hepatitis B as well as obesity and diabetes which contribute to non-alcoholic fatty liver disease. Without the option of liver transplantation, the vast majority of patients with decompensated cirrhosis have a dire prognosis.

Research frontiers

In different parts of the world subsist disparities in access to liver transplantation given the paucity of donor, religious or financial restrictions, access to care, *etc*. Systematic and effective ways to optimize the limited resource of organ donors is essential.

Innovations and breakthroughs

Geographic Information System (GIS) studies the impact of geography on many problems through statistical modeling and analysis. It has been used to guide decisions in business, government, environment, but has yet to be adopted in healthcare. Based on the listing for liver transplantation database from the United States from 2003 to 2012 in one region, GIS revealed clustering of high and low rates of listing for liver transplantation in several geographic areas that could not have otherwise been predicted. This method can be adopted in different parts of the world and contribute to better allocation of resources to decrease the disparities in access to liver transplantation.

Applications

Geography encompasses different variables that can impact access to care and public health outcomes it should be included in the decision making process of allocating resources to decrease disparity and reveal unsuspected variables.

Terminology

United Network for Organ Sharing is a private, non-profit organization that manages the United States organ transplant system under contract with the federal government.

Peer-review

The study was very unique and will be helpful to introduce similar system in the other countries.

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SYSTEMATIC REVIEWS

Non-alcohol fatty liver disease in Asia: Prevention and planning

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Abstract

AIM: To review all of epidemiological aspects of non-alcoholic fatty liver disease (NAFLD) and also prevent this disease is examined.

METHODS: We conducted a systematic review according to the PRISMA guidelines. All searches for writing this review is based on the papers was found in PubMed (MEDLINE), Cochrane database and Scopus in August and September 2014 for topic of NAFLD in Asia and the way of prevention of this disease, with no language limitations. All relevant articles were accessed in full text and all relevant materials was evaluated and reviewed.

RESULTS: NAFLD is the most common liver disorder in worldwide, with an estimated with 20%-30% prevalence in Western countries and 2%-4% worldwide. The prevalence of NAFLD in Asia, depending on location (urban *vs* rural), gender, ethnicity, and age is variable between 15%-20%. According to the many studies in the world, the relationship between NAFLD, obesity, diabetes mellitus, and metabolic syndrome (MS) is quiet obvious. Prevalence of NAFLD in Asian countries seems to be lower than the Western countries but, it has increased recently due to the rise of obesity, type 2 diabetes and MS in this region. One of the main reasons for the increase in obesity, diabetes and MS in Asia is a lifestyle change and industrialization. Today, NAFLD is recognized as a major chronic liver disease in Asia. Therefore, prevention of this disease in Asian countries is very important and the best strategy for prevention and control of NAFLD is lifestyle modifications. Lifestyle modification programs are typically designed to change bad eating habits and increase physical activity that is associated with clinically significant improvements in obesity, type 2 diabetes and MS.

CONCLUSION: Prevention of NAFLD is very important

in Asian countries particularly in Arab countries because of high prevalence of obesity, diabetes and MS.

Key words: Non-alcoholic fatty liver disease; Metabolic risk factors; Asian countries; Prevention

Core tip: Today non-alcoholic fatty liver disease (NAFLD) is one of the main concerns of the medical world. NAFLD is identified as a main risk factor for chronic liver disease across the world. NAFLD is clearly linked with obesity, type 2 diabetes and metabolic syndrome (MS). The prevalence of NAFLD is lower in Asian countries than Western countries but, it has increased dramatically in recent years because of increasing rate of obesity, type 2 diabetes and MS in this region. The high prevalence of obesity with diabetes, and MS would increase the risk of NAFLD in recent years. So, prevention of these factors is the key strategy to reduce the incidence of NAFLD.

Ashtari S, Pourhoseingholi MA, Zali MR. Non-alcohol fatty liver disease in Asia: Prevention and planning. *World J Hepatol* 2015; 7(13): 1788-1796 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i13/1788.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i13.1788

INTRODUCTION

Nowadays, non-alcoholic fatty liver disease (NAFLD) is a major health concern worldwide which is characterized by abnormal fat accumulation in liver cells[1,2]. The development process of NAFLD can be started from simple steatosis (NAFLD) to non-alcoholic steatohepatitis (NASH) and finally leads to cirrhosis and hepatocellular carcinoma in absence excessive alcohol intake[3,4]. NAFLD is one of the main cause of chronic liver disease in industrializes countries[5,6]. According to the American Association for the Study of Liver Disease Guidelines[7], liver biopsy is the gold standard for the diagnosis of NAFLD, nevertheless ultrasonography is more commonly used particularly in developing countries, because of increased health risks and high expenditures associated with liver biopsies[2]. So, the prevalence of NAFLD varies according to the method used to diagnosis and study population[7-9]. In generally, the prevalence of NAFLD ranges is from 6.3% to 33% worldwide, and prevalence of NASH is from 3% to 5% in general population[7,10]. Despite the low prevalence of NAFLD in Asian countries (12%-24%)[11], than in Western countries (> 20%)[12], it is identified as a main risk factor for chronic liver disorder in all over world[13]. In Asian countries, the prevalence of NAFLD varies in different countries, and is related to the age, gender, locality and ethnicity[11]. NAFLD prevalence increases with age[6], and also men (40-49 years) tend to get NAFLD earlier than women (over 50 years)[11,14]. According to the other studies especially in South-East region of Asia[15-18], more men than women had NAFLD. For diagnosis of NASH, liver biopsy is required and it's costly especially in low-income countries so the establish the prevalence of NASH is difficult. More than 30% of obese patients may have NASH and 12%-25% have fibrosis[2,19,20]. In predictors and diagnosis of NASH and fibrosis, diabetes and insulin resistance are the two main factors than body mass index (BMI)[21,22].

MATERIALS AND METHODS

We conducted a systematic review according to the PRISMA guidelines. All searches for writing this review is based on the papers was found in PubMed (MEDLINE), Cochrane database and Scopus in August and September 2014 for topic of NAFLD in Asia and the way of prevention of this disease, with no language limitations. All relevant articles were accessed in full text and all relevant materials was evaluated and reviewed. We extracted data on epidemiology of NAFLD, Burden and prevalence of NAFLD, risk factors characteristics association NAFLD, and prevention of NAFLD. We analyzed the data and reported the results in the tables and text.

RESULTS

Based on systematic reviews, defines NAFLD as a compound disorder delineated by a set of metabolic syndrome (MS) risk factors, usually related to obesity, diabetes, hypertension and dyslipidemia[3,11,23,24]. Insulin resistance is the main factor in NAFLD pathogenesis, because of association between NAFLD and MS[11]. The presence of obesity and type 2 diabetes mellitus (T2DM) significantly increases the risk of NAFLD[11]. Available data from previous studies indicate that the prevalence of NAFLD likely increases 65%-70%[2,25-27] in T2DM populations and greater than 75% and 90% in obese people[28,29] and morbidly obese patients[30,31], respectively. In addition, NAFLD can be increase the risk of cardiovascular events in obese and diabetic people[2,32].

Obesity

Obesity has doubled worldwide since 1980. In Asia also, based on several national health surveys[33-36] prevalence of overweight and obese subjects has increased in the past few decades, but it varies between countries[37] [Table 1: Provides the 2010 World Health Organization (WHO); Global status report on non-communicable disease statistics for overweigh and obesity prevalence in Asian countries, Data adjusted for 2008 for comparability]. The prevalence of obesity in eastern Asia (*e.g.,* China, Japan, South Korea and Taiwan), Southern Asia (*e.g.,* Bangladesh, India, Pakistan and Sri Lanka), and South-Eastern Asia (*e.g.,* Malaysia, Philippines, Singapore, Thailand and Vietnam) is quite low compared with developed countries such as the United States[38-40]. The

Table 1 Prevalence of overweight and obesity in Asian countries, estimates for 2008 (%)

Country	Overweight[1]			Obesity[2]		
	Males	Females	Total	Males	Females	Total
Kuwait	78.4	79.5	78.8	37.5	49.8	42.0
Saudi Arabia	69.1	68.8	69.0	28.6	39.1	33.3
Qatar	73.1	70.2	72.3	31.3	38.1	33.2
Egypt	60.4	75.3	67.9	21.4	44.5	33.1
Bahrain	70.9	70.3	70.6	29.5	38.0	32.9
UAE	71.3	71.2	71.3	30.0	39.0	32.7
Turkey	59.7	64.1	61.9	21.7	34.0	27.8
Lebanon	66.1	57.9	61.8	25.8	29.0	27.4
Iraq	59.5	65.1	62.3	20.6	33.4	27.0
Oman	56.9	54.2	55.8	18.9	23.8	20.9
Iran	46.0	56.8	51.4	12.4	26.5	19.4
Malaysia	42.1	46.3	44.2	10.4	17.6	14.0
Thailand	26.5	37.4	32.2	5.0	12.2	8.8
South Korea	34.3	29.2	31.8	7.2	8.3	7.7
Singapore	33.9	26.4	30.2	7.0	7.1	7.1
Philippines	24.6	28.4	26.5	4.6	8.0	6.3
China	25.5	25.4	25.4	4.7	6.7	5.7
Pakistan	19.1	27.1	23.0	3.3	7.8	5.5
Japan	30.1	19.2	24.4	5.8	4.4	5.0
India	9.9	12.2	11.0	1.3	2.4	1.9
Vietnam	9.5	10.9	10.2	1.2	2.1	1.7
Bangladesh	7.4	7.8	7.6	0.9	1.3	1.1

[1]Overweight: The percentage of the population aged 20 or older having a body mass index (BMI) ⩾ 25 kg/m^2; [2]Obesity: The percentage of the population aged 20 or older having a BMI ⩾ 30 kg/m^2. Adapted from World Health Organization, non-communicable diseases report[41]. UAE: United Arab Emirates.

highest rate of obesity in these regions of Asia are in Malaysia and Thailand, where 14% and 8.8% of adults are reported to be obese, respectively[41]. The lowest obesity rates in these regions are in the less developed parts of Asia: 1.1% in Bangladesh, 1.7% in Vietnam and 1.9% in India[36,41]. In contrast to these regions of Asia, in West Asian countries (Middle East countries; *e.g.,* Iran, Iraq, Bahrain, Egypt, Kuwait, Saudi Arabia, Oman and Qatar) prevalence of obesity is very high and almost is equal with the Western developed countries. So that in countries such as Kuwait (42%), Saudi Arabia (33.3%), Qatar (33.2%) and Egypt (33.1%), the prevalence of obesity is higher than United States (33%)[41]. Except in Japan, Rates of obesity among women are twice that of men in all Asian countries.

NAFLD prevalence is much higher estimates in obese people[42]. Population-based survey from Iran reported that obesity and MS are the most predictive factors of NAFLD[43]. In addition, in the other Population-based study conducted China, the relationship between NAFLD and obesity have been reported so that, among 661 patients with fatty liver, 611 (92%) patients were obese[44]. The high prevalence of obesity in the West of Asia also increases the risk of NAFLD[11].

Diabetes mellitus

Diabetes mellitus is present as one of the biggest public health problems of the recent century[45]. The International diabetes federation (IDF)[46] estimated the global burden diabetes was 382 million (comparative prevalence: 8.3%) in 2013 and it would be likely more than double to 592 million (comparative prevalence: 8.8%) by 2035. Approximately 175 million people worldwide living with diabetes are unaware of their disease[46]. According to the 6th edition of the Diabetes Atlas in 2013[46], Saudi Arabia (24%), Kuwait (23.1%) and Qatar (2.9%) are among the world's top ten countries with the highest prevalence of diabetes in 20-79 years population are in the Middle-East countries. And also from the ten countries with the highest number of diabetic people (20-79 years), five countries are located in Asia that which includes; China, India, Indonesia, Egypt and Japan. T2DM consist 85% to 95% in high-income countries and even higher percentage in low and middle income countries[47]. It is one of the major health problems in the world, and also is known as an important risk factor for NAFLD[48,49]. T2DM prevalence is increasing in the world[50] and also in Asian countries the prevalence rate of it has increased during the past three decades[51]. Increasing the T2DM in Asian countries for the following reasons is different from the countries because of the short time spread, and that can be seen in a younger age group and people with much lower BMI[37]. Many ethnic studies on Asian population pointed out, that they have more abdominal obesity and visceral fat (3%-5%) than other ethnic groups[52-54]. Improper accumulation of fat in abdominal and visceral adiposity can cause to increase hepatic insulin resistance and T2DM, which can cause an abnormal accumulation of fat in the liver[55,56]. This rapidly-growing prevalence of T2DM among the Asian countries is related to the rapid economic developments, aging, urbanization, changes in nutrition, and increases in sedentary lifestyles, and also increases with increasing prevalence of obesity and

Table 2 Prevalence of diabetes in Asian countries, estimates for 2013

Country	Adult population (20-79) in 1000s	Diabetes cases (20-79) in 1000s	Diabetes national prevalence (%)	[1]Diabetes comparative prevalence (%)	Diabetes related deaths (20-79)
Saudi Arabia	18056.84	3650.89	20.22	23.87	22113
Kuwait	2293.74	407.53	17.77	23.09	1122
Qatar	1796.42	282.53	15.73	22.87	651
Bahrain	974.96	168.66	17.30	21.84	706
UAE	7443.81	745.94	10.02	18.98	1385
Egypt	48276.39	7510.60	15.56	16.80	86478
Lebanon	3295.49	478.96	14.53	14.99	6637
Oman	2493.25	199.78	8.01	14.24	1214
Malaysia	18919.44	1913.24	10.11	10.85	24049
Singapore	4058.27	498.19	12.28	10.42	4134
Iran	52145.45	4395.93	8.43	9.94	38002
Iraq	16473.21	1226.22	7.44	9.50	17643
India	760429.73	65076.36	8.56	9.09	1065053
China	1023050.42	98407.38	9.62	9.02	1271003
Yemen	11568.55	708.12	6.12	8.45	9892
Taiwan	17605.38	1721.06	9.78	8.30	-
Afghanistan	12619.61	794.70	6.30	8.27	18864
Pakistan	99369.82	6712.70	6.76	7.90	87354
South Korea	37365.67	3323.90	8.90	7.48	30836
Philippines	54210.53	3256.21	6.01	6.86	54535
Bangladesh	92271.61	5089.04	5.52	6.31	102139
Vietnam	61387.55	3299.11	5.37	5.81	54953
Thailand	49049.75	3150.67	6.42	5.67	66943
Japan	95304.38	7203.78	7.56	5.12	64680

[1]All comparisons between countries should be done using the comparative prevalence, which is adjusted to the world population. Adapted from International Diabetes Institute[46]. UAE: United Arab Emirates.

MS[57,58].

Middle East region, particularly Arab speaking countries have some of the highest rate of diabetes in the world[59]. The prevalence of T2DM has increased dramatically in this region over the last three decades because of industrial development. Most of countries in the Middle East such as Kuwait, Saudi Arabia, Qatar, Bahrain and the United Arab Emirates are the world's leaders in term of T2DM prevalence[60]. In both developed and developing countries diabetes is the main cause of NAFLD, and also the prevalence of NAFLD is higher in people with diabetes than in non-diabetic[61] (Table 2: Provides the 2013 IDF statistics for diabetes prevalence in Asian countries).

MS

MS is known as a collection of interrelated abnormalities that increase the risk of T2DM and NAFLD[62]. According to the available data, experimental and epidemiological studies describe the NAFLD as the hepatic manifestation of MS[63,64]. Today prevalence of MS is increasing and the main risk factors associated with MS are abdominal obesity, hypertension, dyslipdemia, insulin resistance and glycemia intolerance[24]. Different criteria have been introduced in recent years to detect MS. The first criteria definition of MS was published in 1998 by WHO, according to this definition impaired glucose tolerance, and impaired fasting glucose, T2DM or insulin resistance are known as essential components of the MS, along with at least two of the following parameters: hypertension (> 140/90 mmHg), obesity (BMI = 30 kg/m^2), hypertriglyceridemia (≥ 150 mg/dL) or high density lipoprotein cholesterol (HDL-C) values (< 35 in males and < 40 in females) and microalbuminuria (≥ 20 μg/min)[65-67]. On the other hand, in 2001, the National Cholesterol Education Program Adult Treatment Panel Ⅲ (NCEP-ATP Ⅲ) published a new set of criteria of MS that included waist circumference as define obesity (≥ 102 cm in males and ≥ 88 cm in females), arterial hypertension (≥ 130/85 mmHg), fasting glucose(≥ 110 mg/dL) and blood lipids as HDL-C values (< 40 mg/dL in males and < 50 mg/dL in females) and hypertriglyceridemia (≥ 150 mg/dL)[68]. The NCEP-ATP Ⅲ definitions differed from WHO and European Group for the study of Insulin Resistance definitions in that insulin resistance is not necessary for diagnostic. In 2005, the IDF published other criteria to define the MS which proposed abdominal obesity as the essential components of the diagnosis of MS, abdominal obesity (Europe men: ≥ 94 cm, Europe women ≥ 80 cm and for Asian men: ≥ 90 cm, Asian women ≥ 80 cm), arterial hypertension (≥ 130/85 mmHg), fasting glycaemia (≥ 100 mg/dL), HDL-C values (< 40 mg/dL in males and < 50 mg/dL in females) and hypertriglyceridemia (≥ 150 mg/dL)[69-72]. The American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI) published a new set of criteria of MS that abdominal obesity is not required as a risk factor. The definition provided by the AHA/NHLBI of abdominal obesity with IDF guidelines was quite different[70,73]. So, in recent years AHA/NHLBI and IDF offered a new definition of criteria that two side agreed that abdominal obesity is 1 of 5 criteria for identifying

Table 3 American Heart Association/National Heart, Lung and Blood Institute metabolic syndrome diagnostic criteria

Measure	Categorical cut points
Elevated waist circumference[1]	Population and country specific definition
Elevated triglycerides	⩾ 150 mg/dL (1.7 mmol/L) or drug treatment for high triglycerides (*i.e.*, fibrates or nicotinic acid)
Low HDL-C[2]	< 40 mg/dL (1.0 mmol/L) in males < 50 mg/dL (1.3 mmol/L) in females Or drug treatment for low HDL-C (*i.e.*, fibrates or nicotinic acid)
Elevated blood pressure	Systolic ⩾ 130 mmHg Diastolic ⩾ 85 mmHg Or drug treatment for hypertension
Elevated fasting glucose	⩾ 100 mg/dL Or drug treatment for elevated glucose

[1]Waist circumference for abdominal obesity by different organization for each population or country specific: (1) Asian (WHO) ⩾ 90 cm men or ⩾ 80 cm women; (2) Japanese (Japanese obesity society) ⩾ 85 cm men or ⩾ 90 cm women; (3) China (Cooperative Task Force) ⩾ 85 cm men or ⩾ 80 cm women; (4) Mediterranean and Middle East (Arab) population (IDF) ⩾ 94 cm men or ⩾ 80 cm women; (5) United States (AHA/NHLBI) ⩾ 102 cm men or ⩾ 88 cm women; (6) South and Central American (WHO) ⩾ 90 cm men or ⩾ 80 cm women; (7) European (European Cardiovascular Societies) ⩾ 102 cm men or ⩾ 88 cm women; and (8) Sub-Saharan African (IDF) ⩾ 94 cm men or ⩾ 80 cm women; [2]High density lipoprotein cholesterol indicates high-density lipoprotein cholesterol. Adapted from Alberti *et al*[74]. AHA/NHLBI: American Heart Association/National Heart, Lung and Blood Institute; WHO: World Health Organization; IDF: International diabetes federation.

but is not essential for diagnosis[74] (Table 3: Provides the criteria for clinical diagnosis of the MS).

Prevalence of MS varies and depends on the criteria used in different definitions[75,76]. And it is increasing in different region like Asia[77] and developing countries[78], it has been reported 12.8% to 41.1% in different part of the world[79]. Prevalence of MS depends on criteria used is different for example the IDF guidelines with a lower abdominal obesity cut-off (90 cm for men, 80 cm for women) identify a greater prevalence of MS than the NCEP-ATP Ⅲ[80-83]. In 2007, the prevalence of MS in the Iran was reported by IDF and ATPⅢ criteria 32.1% and 33.2% respectively[84]. According to 2005 version of IDF criteria, China[67], Taiwan[85,86], Hong Kong[87], and Thailand[88] had prevalence rates ranging between 10%-15% (in 2008). On the other hand, rates for Koreans[89], approximately one quarter, were higher than the Chinese and Thais. India[90] had significantly high prevalence rates compared to the rest of Asia. Unfortunately, no many studies have been done in the field of MS in Arab countries[81]. Because of increasing prevalence of obesity and diabetes in Middle-East countries particularly in Arab countries, increased risk of MS is high[91,92].

The major reason for the higher rate using in the new definition is because of focus on abdominal obesity, which is the most common component in Arab countries[81]. The increased prevalence of MS was shown in both genders, whereas the increased prevalence is higher in women in Arab populations[91,93]. And also the other components of the MS, diabetes is more common among the Arab population than other regions of the world and is estimated to have increased rapidly in the region[81,91]. Approximately 50% of patients with T2DM also suffer from MS, whereas the risk of NAFLD in these patients is higher more than the other persons[94].

DISCUSSION

Due to the increasing rate of NAFLD, prevention of this is one of the most important issues of the world. Prevention methods of NAFLD that is limited to the prevention of risk factors, because the pathogenesis of this disease is unknown. So prevention of the risk factors of NAFLD such as obesity, insulin resistance, T2DM and MS is the key strategy to reduce the incidence rate of NAFLD in the world[95]. Today, due to drastic changes in lifestyle and desire to in sedentary lifestyle, because of rapid economic and social changes in many countries, including Asian countries, prevalence of obesity, T2DM and MS are on the rise, which are important risk factors for NAFLD.

Hence, the key management of NAFLD is lifestyle modifications. Lifestyle modification programs are typically designed to change bad eating habits and increase physical activity that is associated with clinically significant improvements in obesity, T2DM and MS. Many studies indicate that lifestyle modification, including a reduction in intake of saturated fat and refined carbohydrates and sweetened beverages, may reduce aminotransferases and improve hepatic steatosis[96-99]. Earlier studies suggested that reduction of body weight by 10% can normalize liver test, but recent studies have shown that loss of at least 3%-5% of body weight can achieve improvement in hepatic steatosis[100,101]. Control and reduce the incidence of insulin resistance and MS is another important aspect of prevention and management of NAFLD[7,14]. Early detection, appropriate treatment, and also care programs with essential training can be an effective step in control and reduce the incidence of MS, insulin resistance and also cardiovascular disease and diabetes. Not only Lifestyle changes, weight loss and regular physical activity are essential first steps for the prevention and treated patients with NAFLD, but also the prevention of metabolic risk factors, such as diabetes, dyslipidemia, hypertension is also very important[4]. However, in addition to lifestyle changes for the treatment of patients with NAFLD, are there specific pharmacologic therapies such as insulin sensitizers (metformin and thiazolidinediones)[102-105], weight loss drugs (orlistat and sibutramine)[106], antioxidants (vitamin E)[107], and have also considered bariatric surgery for morbidly obese patients[4,108].

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COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) is a major health concern worldwide which is characterized by abnormal fat accumulation in liver cells. Today, NAFLD is identified as a main cause of chronic liver disease in Asia. Due to the increasing rate of NAFLD, prevention of this is one of the most important issues of the world. Prevention methods of NAFLD that is limited to the prevention of risk factors, because the pathogenesis of this disease is unknown.

Research frontiers

The objective of this study was to review systematically all of aspects of NAFLD in Asia, provides updated epidemiological data on NAFLD and its etiology and also this study has examined the current and future possibilities of prevention of this disease in Asian countries.

Innovations and breakthroughs

Based on systematic reviews, NAFLD is tightly linked with obesity, type 2 diabetes mellitus (T2DM) and the presence of metabolic syndrome (MS). Because of increasing prevalence of obesity, T2DM and MS in Asian countries particularly in Arab countries, increased risk of NAFLD is high in this region. So, by increasing the prevalence and incidence of NAFLD in this region prevention of this disease is very important.

Applications

Prevention of NAFLD should be considered in the Asian countries, because it is increasingly recognized as a major chronic liver disease in these regions.

Terminology

NAFLD is characterized by abnormal fat accumulation in liver cells. The development process of NAFLD can be started from simple steatosis (NAFLD) to non-alcoholic steatohepatitis and finally leads to cirrhosis and hepatocellular carcinoma, in absence excessive alcohol intake.

Peer-review

This is a well-written and comprehensive review of the epidemiology of nonalcoholic fatty liver disease in Asia.

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META-ANALYSIS

Transjugular intrahepatic portosystemic stent shunt for medically refractory hepatic hydrothorax: A systematic review and cumulative meta-analysis

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Abstract

AIM: To assess the effectiveness of transjugular intrahepatic portosystemic stent shunt (TIPSS) in refractory hepatic hydrothorax (RHH) in a systematic review and cumulative meta-analysis.

METHODS: A comprehensive literature search was conducted on MEDLINE, EMBASE, and PubMed covering the period from January 1970 to August 2014. Two authors independently selected and abstracted data from eligible studies. Data were summarized using a random-effects model. Heterogeneity was assessed using the I^2 test.

RESULTS: Six studies involving a total of 198 patients were included in the analysis. The mean (SD) age of patients was 56 (1.8) years. Most patients (56.9%) had Child-Turcott-Pugh class C disease. The mean duration of follow-up was 10 mo (range, 5.7-16 mo). Response to TIPSS was complete in 55.8% (95%CI: 44.7%-66.9%), partial in 17.6% (95%CI: 10.9%-24.2%), and absent in 21.2% (95%CI: 14.2%-28.3%). The mean change in hepatic venous pressure gradient post-TIPSS was 12.7 mmHg. The incidence of TIPSS-related encephalopathy was 11.7% (95%CI: 6.3%-17.2%), and the 45-d mortality was 17.7% (95%CI: 11.34%-24.13%).

CONCLUSION: TIPSS is associated with a clinically relevant response in RHH. TIPSS should be considered early in these patients, given its poor prognosis.

Key words: Cirrhosis; Portal hypertension; Hepatic hydrothorax; Transjugular intrahepatic portosystemic stent shunt; Meta-analysis

Core tip: Evidence on the effectiveness of transjugular intrahepatic portosystemic stent shunt (TIPSS) in patients with refractory hepatic hydrothorax (RHH) is scarce and variable. This paper summarizes available data on the effectiveness of TIPSS in RHH in a cumulative meta-analysis. The sum total of the evidence shows that TIPSS is associated with a clinically relevant response in three-quarters of patients with medically RHH. We suggest that TIPSS be considered early in patients with RHH, given its impact on quality of life and prognosis. However, caution should be exercised in older patients and those with severe underlying liver or renal dysfunction.

Ditah IC, Al Bawardy BF, Saberi B, Ditah C, Kamath PS. Transjugular intrahepatic portosystemic stent shunt for medically refractory hepatic hydrothorax: A systematic review and cumulative meta-analysis. *World J Hepatol* 2015; 7(13): 1797-1806 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i13/1797.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i13.1797

INTRODUCTION

Hepatic hydrothorax (HH) is the accumulation of significant pleural effusion, usually in excess of 500 mL, in a patient with cirrhosis without coexisting primary cardiopulmonary disease[1-3]. It is a relatively uncommon complication of end-stage liver disease, with an estimated prevalence among cirrhotic patients of 5% to 10%[1,3-5]. Although the exact mechanisms involved in the development of HH have not been completely elucidated, the most widely accepted mechanism is the passage of fluid from the peritoneal to the pleural cavity through diaphragmatic defects, usually less than 1 cm in diameter[6-9]. The one way flow of the ascitic fluid into the pleural cavity is also thought to be influenced by the negative intrathoracic pressure. The effusion, typically a transudate, most commonly occurs in the right hemithorax (85%)[3,10]. Ascites can be absent in up to 20% of patients with HH[11-13]. A diagnostic thoracentesis often confirms diagnosis and excludes infection.

The initial management of HH is similar to that for ascites. Maximal sodium restriction (< 70-90 mmol/d) and optimal tolerated diuretics are the first-line therapy. Therapeutic thoracentesis is a safe and effective way to rapidly relieve symptoms of dyspnea in patients with large effusions (1.5-2.0 L)[5]. However, when thoracentesis is required more than once every 2 to 3 wk in patients on maximal sodium restriction and optimal diuretics, it is considered refractory, and alternative treatments should be considered. Pleurodesis and peritoneovenous shunts are surgical options that are usually associated with rapid fluid reaccumulation and procedure-related complications, and they are not generally recommended as treatments for HH[14,15]. In the absence of a large pneumothorax, hemothorax, or frank empyema, a chest tube should not be inserted in patients with HH[16,17].

Up to 25% of patients with HH will become refractory to treatment[18], compared to only 10%[17] of patients with cirrhotic ascites. Refractory HH (RHH) has traditionally been associated with poor prognosis. Patients with RHH should therefore be considered for liver transplantation. The treatment strategies for RHH are similar but not identical to those for refractory ascites. In patients with prerenal azotemia, therapeutic thoracentesis as a long-term regular treatment is not recommended because of the risk for bleeding and pneumothorax[6]. Transjugular intrahepatic portosystemic stent shunt (TIPSS) is a nonsurgical, angiographic technique of reducing hepatic sinusoidal pressure, which then results in a reduction in the accumulation of fluid in the peritoneal and pleural space. The procedure is often used as a bridge to liver transplantation in patients with end-stage liver disease. Since RHH is an uncommon complication of cirrhosis, most of the studies on the effectiveness of TIPSS have been limited to small numbers of patients, primarily in the form of case reports[19-22] or case series[3,14,23-28]. Findings from these studies have varied substantially. The purpose of this study was to evaluate the effectiveness of TIPSS in patients with RHH by pooling all available evidence in a systematic review with cumulative meta-analysis.

MATERIALS AND METHODS

Literature search

A comprehensive literature search was conducted using Ovid on MEDLINE and EMBASE, PubMed Cochrane Library, and the Web of Science for the period from January 1970 to August 2014. The search terms included, in different combinations: "portosystemic shunt", "transjugular intrahepatic stent shunt", "liver cirrhosis or end-stage liver disease", "hydrothorax", "pleural effusion" and "ascites". The search was limited to studies in humans published in English. References of articles meeting inclusion criteria and review articles on the subject were manually searched for other relevant studies that might have been missed.

Selection of articles

The selection criteria were studies in: (1) patients with cirrhosis irrespective of etiology; (2) patients with medically RHH with or without ascites; and (3) series that included at least 10 patients. Case reports or series with fewer than 10 patients were excluded. Two reviewers (ICD and BFAB) independently screened article titles and abstracts for selection. Once unrelated articles were excluded, each eligible article was then reviewed in full.

Data extraction

Data were abstracted by the same 2 investigators onto standardized paper forms and entered into an Excel

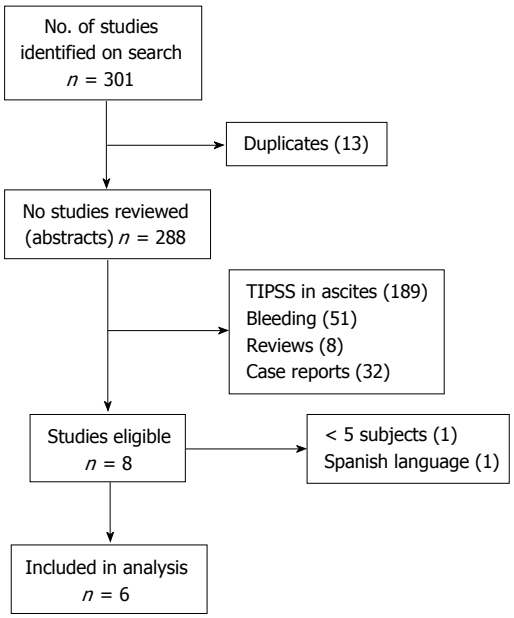

Figure 1 Study selection flow chart. Of a total of 301 studies that included at least 10 patients in the series, only 6 studies met selection criteria. TIPSS indicates transjugular intrahepatic portosystemic stent shunt. TIPSS: Transjugular intrahepatic portosystemic stent shunt.

spreadsheet (Microsoft Corp, Redmond, Washington). The following information were abstracted from each study: author, time period of study, study methods and participants, outcome of interest [mortality/survival, response to TIPSS, TIPSS-related complications, incidence of hepatic encephalopathy (HE), mean change in hepatic venous pressure gradient (HVPG), and country of study]. Differences between the 2 abstracting investigators were settled by reviewing the article together and seeking an independent input from a third investigator (BS).

Definition of operational variables

Medically RHH: Patients with underlying liver cirrhosis who underwent TIPSS because of symptomatic HH that had failed to respond to sodium (< 2 g/d) restriction, who had optimal diuretics dosing (maximal tolerated doses without electrolyte abnormalities or clinically significant side effects), and who required frequent (more than once every 2-3 wk) thoracentesis were classified as having medically RHH.

Response to TIPSS

Response to TIPSS was based on clinical or radiographic evidence of hydrothorax post-TIPSS. Response was categorized as complete, partial, or absent. Response was classified as complete if the patients' symptoms of shortness of breath resolved or returned to baseline, with no evidence of pleural effusion requiring thoracentesis. Partial response was defined as improvement of shortness of breath but without complete symptomatic resolution; thoracentesis was required less frequently than pre-TIPSS. Absent response was defined as persistent or worsening symptoms of shortness of breath and/or persistent need for thoracentesis. Radiologically, complete response was defined as undetectable pleural effusion on chest radiographs, computed tomogram, or ultrasonogram; partial response if pleural effusion decreased compared to pre-TIPSS; and absent response if pleural effusion was unchanged or increased. The studies used either radiologic and/or clinical criteria to assess response to TIPSS.

TIPSS-related complications: (1) HE. TIPSS related HE was defined as new onset (*i.e.*, never existed prior to TIPSS) or worsening (increased in frequency or severity of encephalopathy, compared to pre-TIPSS status). One study considered HE as TIPSS related if it occurred within 30 d of the procedure[24]; and (2) Mortality After TIPSS. Death was evaluated as early (*i.e.*, occurred within 45 d of the procedure) and overall (death irrespective of when the event occurred throughout the follow-up period). The follow-up period varied across the studies, with the longest duration being 5 years.

Statistical analysis

Data from eligible studies were pooled using a random-effects model with Stata version 11 (Stata Corp LP, College Station, Texas). Outcomes are expressed as proportions (percentages) with 95%CIs. The pooled analyses are presented as forest plots. Since there were only 6 eligible studies, we determined a priori that subgroup analyses would not be performed. Statistical heterogeneity between studies was assessed using the Cochran Q test and the I^2 statistic. An I^2 value of greater than 50% or a P value of less than 0.05 for the Q statistic was taken to indicate significant heterogeneity. All analyses were performed in accordance with the Meta-analysis of Observational Studies in Epidemiology guidelines (Table 1)[29]. Since this was a cumulative meta-analysis, publication bias was not assessed.

RESULTS

Literature search results

Six studies involving a total of 198 patients were included in the analyses. Two studies were excluded because each had a small number of study subjects and were judged by 2 of the reviewing authors to be of poor quality[3,25]. Figure 1 summarizes the results of the literature search, including the reasons for the exclusion of studies, and Table 2 summarizes the characteristics of the 6 studies that were included in the analysis.

Characteristics of study participants

The mean (SD) age of the 198 patients was 56 years (1.8 years) and 52% were male. The majority of patients had Child class C disease (56.9%), while 40.7% and 0.8% were Child class B and A, respectively. The mean pre- and post-TIPSS HVPG values were 20.14 mmHg (range, 17.4-26.0 mmHg) and 7.37 mmHg (range, 5.7-10.0 mmHg), respectively. The mean duration of follow-up was 10 mo (5.7-16.0 mo). Table 3 shows the results of

Table 1 Checklist summarizing compliance with meta-analysis of observational studies in Epidemiology Guidelines

MOOSE criteria[a]	Met (yes/no)
Reporting background should include	
Problem definition	Yes
Hypothesis statement	No
Description of study outcome(s)	Yes
Type of exposure or intervention used	Yes
Type of study designs used	Yes
Study population	Yes
Reporting of search strategy should include	
Qualifications of searchers (*e.g.*, librarians and investigators)	Yes
Search strategy, including time period included in the synthesis and keywords	Yes
Effort to include all available studies, including contact with authors	Yes
Databases and registries searched	Yes
Search software used, name and version, including special features used (*e.g.*, explosion)	Yes
Use of hand searching (*e.g.*, reference lists of obtained articles)	Yes
List of citations located and those excluded, including justification	Yes
Method of addressing articles published in languages other than English	Yes
Method of handling abstracts and unpublished studies	No
Description of any contact with authors	No
Reporting methods should include	
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Yes
Rationale for the selection and coding of data (*e.g.*, sound clinical principles or convenience)	Yes
Documentation of how data were classified and coded (*e.g.*, multiple raters, blinding, and interrater reliability)	Yes
Assessment of confounding (*e.g.*, comparability of cases and controls in studies where appropriate)	No
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Yes
Assessment of heterogeneity	Yes
Description of statistical methods (*e.g.*, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	Yes
Provision of appropriate tables and graphics	Yes
Reporting of results should include	
Graphic summarizing individual study estimates and overall estimate	Yes
Table giving descriptive information for each study included	Yes
Results of sensitivity testing (*e.g.*, subgroup analysis)	No
Indication of statistical uncertainty of findings	Yes
Reporting of discussion should include	
Quantitative assessment of bias (*e.g.*, publication bias)	NA
Justification for exclusion (*e.g.*, exclusion of non-English-language citations)	Yes
Assessment of quality of included studies	Yes
Reporting of conclusions should include	
Consideration of alternative explanations for observed results	Yes
Generalization of the conclusions (*e.g.*, appropriate for the data presented and within the domain of the literature review)	Yes
Guidelines for future research	Yes
Disclosure of funding source	Yes

[a]Adapted from Stroup *et al*[29]. Used with permission. MOOSE: Meta-analysis of Observational Studies in Epidemiolgy; NA: Not applicable.

the various outcomes of the individual studies.

RHH response to TIPSS, post-TIPSS mortality, and incident HE

Response to TIPSS was complete in 55.8% (95%CI: 44.7%-66.9%) (Figure 2A) and partial in 17.6% (95%CI: 10.9%-24.2%) of patients (Figure 2B). There was absent response in 21.2% (95%CI: 14.2%-28.3%) of the patients (Figure 2C). There was no evidence of heterogeneity among the 6 studies (P = 0.99, P = 0.65, and P = 0.76) respectively.

Mortality within 45 d (early mortality) of TIPSS placement was 17.74 (95%CI: 11.34%-24.13%) (Figure 3A), while the overall mortality post-TIPSS was 50.17% (95%CI: 39.63%-60.71%) (Figure 3B). Predictors of mortality included older age, severity of liver disease, elevated creatinine and nonresponse to TIPSS. There was no evidence of heterogeneity among the studies (P = 0.86 and P = 0.81, respectively).

The incidence of post-TIPSS encephalopathy was 11.7% (95%CI: 6.3%-17.2%) (Figure 4). On this outcome, however, there was evidence of significant heterogeneity among the studies (P = 0.04).

DISCUSSION

This study shows that TIPSS relieves symptoms in close to three-fourths (73%) of patients with RHH. The 45-d mortality and the 1-year survival in patients with RHH are comparable to those seen in patients with refractory ascites and variceal hemorrhage. The most important predictors of poor outcomes after TIPSS for RHH include older age and severe underlying liver disease and/or associated renal dysfunction.

Table 2 Characteristics of 6 studies evaluating the effectiveness of transjugular intrahepatic portosystemic stent shunt in patients with refractory hepatic hydrothorax

Ref.	Methods and patients	Outcomes/complications	Remarks
Gordon *et al*[14]	Retrospective chart review of 24 consecutive patients with medically RHH	Post-TIPSS response was categorized as complete, partial, or absent	11 patients had variceal bleeding > 4 wk before TIPSS
	Post-TIPSS patients underwent Doppler US studies every 3 to 6 mo	Mean change in HVPG	Stent revision if decreased flow noted
	Mean follow-up was 7.2 mo (range, 0.25-49.0 mo)	TIPSS patency was assessed by change in CTP score, survival, and new or worsened HE	5 failures were CTP C
	Patients with infection were excluded		12 patients had medically RHH; the rest of the 9 patients had TIPSS and RHH as a secondary indication with the primary indication being intractable ascites (n = 7) and gastric varices (n = 2)
Jeffries *et al*[24]	Retrospective chart review of 12 consecutive patients with medically RHH	Post-TIPSS response at ⩽ 1 or > 1 mowas categorized as complete, partial, or absent	Immediate pre- and post-TIPSS prophylactic antibiotics given
	Post-TIPSS, patients had Doppler US studies every 3 mo	TIPSS-related complications: ⩽ 30 and > 30 d	Shunt thrombosis or decreased velocities requiredangioplastic revision
	Mean follow-up was 173 d (range, 7-926 d)	New-onset or worsened HE survival	4 patients had shunt revisions
	Patients with heart failure, HCC, alcoholic hepatitis, or intrinsic renal disease were excluded	Mean change in HVPG	Patients who died or underwent transplant ⩽ 30 d after TIPSS were classified as nonresponders to TIPSS
Siegerstetter *et al*[26]	Retrospective chart review of 40 consecutive patients with medically RHH	Post-TIPSS response was categorized as complete, partial, or absent	8 patients had no ascites; RHH was diagnosed by intraperitoneal methylene blue injection or technetium-Tc-99
	Post-TIPSS, patients had Doppler US studies at 4 wk, then every 3 mo		2 stent size reductions due to chronic HE
	Mean (SD) follow-up was 14 mo [14 (range, 1-54 mo)]	Predictors of survival: Mean change in HVPG New-onset or worsened HE CTP score improvement Survival at 1 yr	
	Patients with infection were excluded		
Spencer *et al*[27]	Retrospective chart review of 21 consecutive patients with medically RHH	30-d mortality	Prophylactic antibiotics administered
	Post-TIPSS, patients had Doppler US studies at 1, 3, and 6 mo, then every 6 mo	Post-TIPSS complications: Early (⩽ 30 d) or late(> 30 d)	Radiographic and clinical response
	Mean follow-up was 223 d	New-onset or worsened HE	TIPSS placement 100% successful
	Patients with severe right-sided heart failure and patients with PVT with cavernous transformation were excluded	Post-TIPSS response was categorized as complete, partial, or absent	1 patient with a partial response was weaned off oxygen due to decreased pleural fluid
		Mean change in HVPG Cumulative survival	
Wilputte *et al*[28]	Retrospective chart review of 28 consecutive patients with medically RHH	Mean change in HVPG	Stent revised for stenosis, obstruction, or relapsing RHH
	Post-TIPSS, patients had Doppler US at 24 h and at 1, 2, 3, 6, 9, and 12 mo, then every 6 mo	30-d mortality post-TIPSS	Patients who underwent transplant were censored at surgery date
	Mean (SD) follow-up was 358 d (121 d); 3 patients were excluded due to grade 3 HE, HCC, cardiopulmonary disease, and infection	Response to TIPSS was categorized as complete, partial, and absent	6 patients required TIPSS revision
			2 patients had TIPSS reduction due to intractable HE
			Both covered and uncovered stents were used
Dhanasekaran *et al*[23]	Retrospective chart review of 73 consecutive patients with medically RHH	Post-TIPSS response at 1 mo and 6 mo was categorized as complete, partial, or absent	TIPSS catheterization used if stenosis suspected or RHH reaccumulated
	Patients had Doppler US every 3 mo for 12 mo, then annually	Evaluated predictors of response to TIPSS	Angioplasty performed, if needed
	Patients with heart failure, pulmonary disease, infection, severe HE, portal vein thrombosis, and multiple hepatic cysts were excluded	Assessed for new or worsening HE	Uncovered and covered stents used
		Mean change in HVPG Overall and 30-d mortality	

CTP: Child-Turcotte-Pugh; HE: Hepatic encephalopathy; HCC: Hepatocellular carcinoma; HVPG: Hepatic venous pressure gradient; PVT: Portal vein thrombosis; RHH: Refractory hepatic hydrothorax; TIPSS: Transjugular intrahepatic portosystemic shunt; US: Ultrasound.

Table 3 Summary of studies included in the pooled analyses of transjugular intrahepatic portosystemic shunt in patients with refractory hepatic hydrothorax

Ref.	No. of patients	Complete response (%)	Partial response (%)	45-d mortality (%)	1-yr survival (%)	Predictors of mortality
Gordon *et al*[14]	24	58	21	21	NA	TIPSS nonresponse CTP class C
Jeffries *et al*[24]	12	42	17	25	NA	Age > 65 yr
Siegerstetter *et al*[26]	40	53	28	13	64	Age > 60 yr
Spencer *et al*[27]	21	57	10	29	NA	Medical comorbidities
Wilputte *et al*[28]	28	57	11	14	41	CTP score > 10 Mayo score > 1.5
Dhanasekaran *et al*[23]	73	59	21	19	48	MELD > 15 Nonresponse Elevated creatinine

CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; NA: Not applicable; TIPSS: Transjugular intrahepatic portosystemic stent shunt.

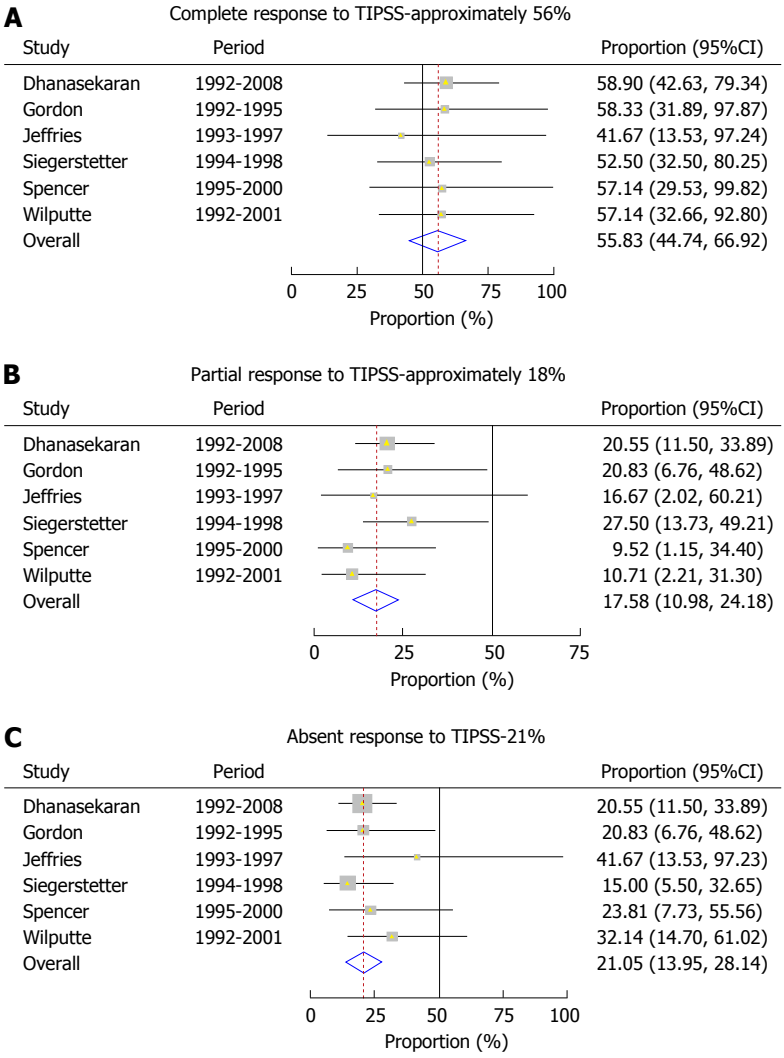

Figure 2 Response to transjugular intrahepatic portosystemic stent shunt. A: Forest plot shows that most [55.8% (95%CI: 44.7%-66.9%)] of the 198 patients in the 6 studies had a complete response (resolution of refractory hepatic hydrothorax without further need for thoracentesis) after TIPSS. There was no evidence of heterogeneity among studies (P = 0.99); B: About one-fifth [17.6% (10.9%-24.2%)] of the patients had only a partial response (defined as improvement in refractory hepatic hydrothorax symptoms and/or a decrease for the need for thoracentesis). There was no evidence of heterogeneity among studies (P = 0.65); C: Just over one-fifth (21.2%) of the patients had no improvement in refractory hepatic hydrothorax after TIPSS. There was no evidence of heterogeneity among studies (P = 0.76). TIPSS indicates transjugular intrahepatic portosystemic stent shunt.

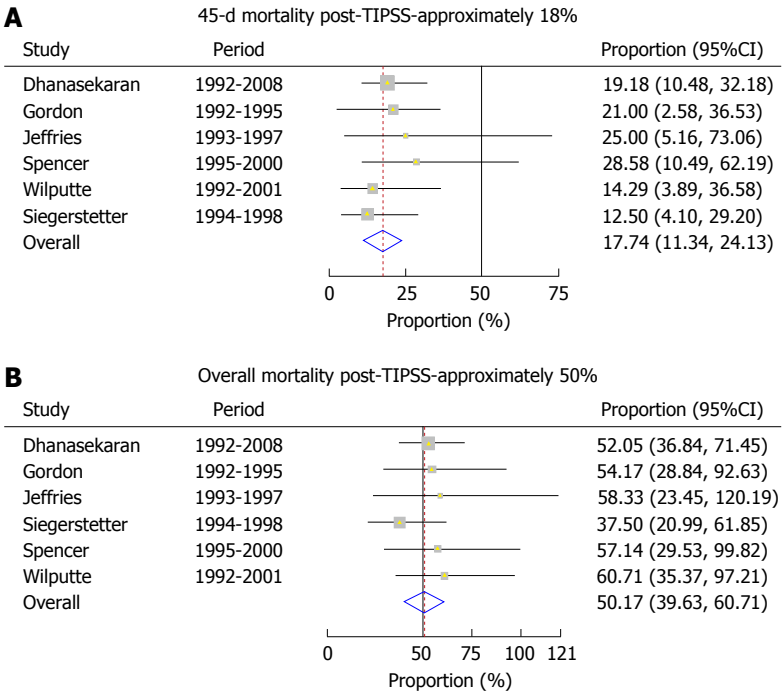

Figure 3 Mortality after transjugular intrahepatic portosystemic stent shunt. A: Forest plot shows that about one-fifth [17.74% (95%CI: 11.34%-24.13%)] of the 198 patients in the 6 studies died within 45 d of undergoing TIPSS. There was no evidence of heterogeneity among studies (P = 0.86); B: Overall mortality after TIPSS was 50.17% (95%CI: 39.63%-60.71%) at a maximum follow-up of 5 years. There was no evidence of heterogeneity among studies (P = 0.81). TIPSS indicates transjugular intrahepatic portosystemic stent shunt.

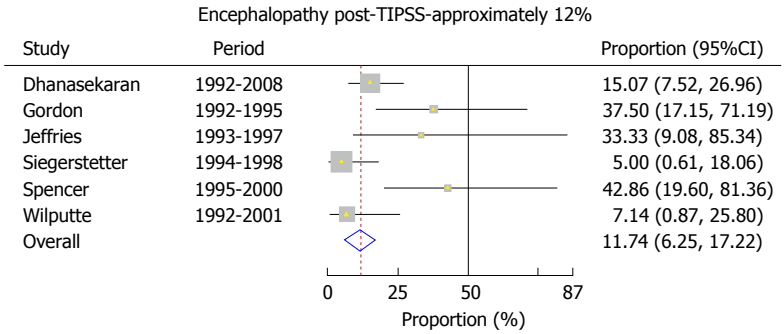

Figure 4 Encephalopathy after transjugular intrahepatic portosystemic stent. TIPSS-related hepatic encephalopathy (new onset or worsening from baseline) was noted in 11.7% (95%CI: 6.3%-17.2%) of the 198 patients in the 6 studies. There was, however, evidence of heterogeneity among the studies (P = 0.04). TIPSS indicates transjugular intrahepatic portosystemic stent shunt.

HH remains a rare complication of liver cirrhosis, with limited therapeutic options. When symptomatic HH fails to respond to medical treatment, repeat thoracentesis is often undertaken. Although thoracentesis is less invasive than TIPSS and is effective in quickly relieving symptoms of dyspnea, it can be associated with complications such as re-expansion pulmonary edema, pneumothorax, and empyema[5,30]. Repeated thoracentesis is also associated with deteriorating clinical status and poor quality of life[1,6]. TIPSS is a nonsurgical approach that decompresses the portal system, thereby addressing the mechanism of fluid collection in the abdomen and/or chest[31]. TIPSS is superior to other treatment modalities in the prevention of rebleeding from varices, and its control of refractory ascites has been well studied in controlled trials[32-36]. In contrast, controlled studies on its use in patients with RHH are lacking, and comparative studies with other treatment options may not be feasible[37,38]. Consequently, evidence on the effectiveness of TIPSS in RHH has been limited to case series with often small numbers of study participants. Results from the 6 studies included in this pooled analysis found a wide range of responses and complication rates, perhaps due to the lack of statistical power. In this study, we combined data from all the small studies, which allowed us to provide the best evidence on TIPSS effectiveness in RHH.

One-fifth of the patients died in the first 45 d after TIPSS placement. This number is well within the range for mortality following TIPSS use in patients with refractory ascites and variceal bleeding[39-44]. Early mortality was observed in patients who developed progressive liver failure, sepsis, renal failure, bleeding,

cardiac complications, and pulmonary complications. Pre-TIPSS factors associated with post-TIPSS mortality included older age, severe liver disease as measured by the Child-Turcott-Pugh score, and renal dysfunction. Ideally, patients with a high likelihood of decompensation after TIPSS should also initiate evaluation for liver transplantation, with TIPSS serving only as a bridge. In a meta-analysis of individual patient data, Salerno *et al*[45] also found that a model composed of age (< 60 years), bilirubin (< 3 mg/dL), and sodium level reliably predicted successful outcomes after TIPSS placement in patients with refractory ascites.

Another important outcome of this study was estimating the incidence of TIPSS-related HE. HE has been shown to predict mortality after TIPSS placement, with survival decreasing from 8 years to about 2 years[46]. The overall incidence of TIPSS-related HE was noted to be 12%. This rate falls within the rate of HE observed with TIPSS for established indications[34,35,47]. The heterogeneity noted between the studies on HE incidence highlights the fact that its diagnosis is subjective.

These results should be interpreted bearing in mind the following: First, this summative analysis was based purely on the published medical literature. We did not have access to individual patient data, which could have allowed us to perform more detailed analysis, especially on factors associated with response to TIPSS and survival (*e.g.*, acute liver failure and procedure related complications). Second, contrary to the extensive literature on refractory ascites, there is a complete lack of controlled trials comparing TIPSS to other therapeutic options for RHH. Conducting a randomized controlled trial on RHH is not feasible because of its relative rarity, and a step-up approach in management is often preferred by clinicians. Most of the 6 studies did not have information on what type of stents were used. Dhanasekaran *et al*[23] compared patients with covered and uncovered stents in a subgroup analysis and found no significant difference in survival, although the patients with covered stents had longer patency rates. Perhaps the small number of patients with covered stents in that study led to the non-significant result. It has been reported that patients who receive covered stents have better outcomes than those who receive uncovered stents[48].

To our knowledge, this is the first ever pooled analysis on TIPSS in patients with RHH. By combining data from all available studies, we were able to present the best evidence on the effectiveness of TIPSS in RHH. We showed that TIPSS is a reasonable therapeutic option in patients with RHH. It is associated with a clinically relevant response in close to three-fourths of patients with RHH. The incidence of TIPSS-related complications in RHH is similar to that observed with other established indications for TIPSS. We suggest that TIPSS should be considered relatively early in patients with RHH, given their poor prognosis. However, caution should be exercised in older patients and in those with severe underlying liver or renal dysfunction.

COMMENTS

Background

Hepatic hydrothorax (HH) which is the accumulation of "ascitic fluid" in the pleural cavity is an uncommon complication of cirrhosis with poor prognosis. When HH fils to respond to traditional medical management (salt restriction and diuretics), it is referred to as refractory HH (RHH).

Research frontiers

Therapeutic options for RHH are limited. Transjugular intrahepatic porto-systemic shunt (TIPSS) has been proposed as an option for RHH. Because HH is rare, studies on the effectiveness of TIPSS in RHH have been restricted to small numbers of patients and findings have varied substantially and are controversial.

Innovations and breakthroughs

The purpose of this study was to evaluate the effectiveness of TIPSS in patients with RHH by pooling all available evidence in a systematic review and cumulative meta-analysis. By combining data from all available studies, the authors generated enough statistical power to study the clinical effectiveness of TIPSS in RHH.

Applications

This study shows that TIPSS leads to a clinically relevant response in about three-fourths (73%) of patients with RHH. The 45-d mortality and the 1-year survival in patients with RHH are comparable to those seen in patients with refractory ascites and variceal hemorrhage. The most important predictors of poor outcomes after TIPSS for RHH include older age and severe underlying liver disease and/or associated renal dysfunction. The authors suggest that TIPSS should be considered early in patients with RHH.

Terminology

HH is the accumulation of fluid in the pleural cavity in patients with cirrhosis. The most widely accepted mechanism for HH is the passage of fluid from the peritoneal to the pleural cavity through a diaphragmatic defect. When HH fails to respond to medical management including salt restriction and maximal tolerated diuretics, it is considered refractory. Transjugular intrahepatic porto-systemic shunt decompresses the portal system, thereby addressing the mechanism of fluid collection in the abdomen and/or chest.

Peer-review

The manuscript is very well written.

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