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* Ishak modified HAI (hepatic activity index) scoring system
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REFERENCES
2. Besivo Phase III Clinical Trial. Protocol No. ID_BVCL011 Clinical Study Report
Aims and Scope

Clinical and Molecular Hepatology (Clin Mol Hepatol) is an international, peer-reviewed, open-access journal published quarterly in English. Clin Mol Hepatol aims to share advanced and latest knowledge, trend, and understanding of hepatobiliary diseases, to provide a wide open academic forum for active debate and discussion among clinical doctors, translational researchers, and basic scientists, and to improve public health through a multidisciplinary approach, especially in resource-limited Asia-Pacific area with high prevalence of B viral infection and hepatocellular carcinoma. In addition, Clin Mol Hepatol gives priority to epidemiological studies of hepatobiliary diseases in East Asia, North Asia, Southeast Asia, Central Asia, South Asia, Southwest Asia, Pacific, Africa, Central Europe, Eastern Europe, Central America, and South America.

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Clinical and Molecular Hepatology 2020;26:83-127

Guideline

KASL clinical practice guidelines for liver cirrhosis: Varices, hepatic encephalopathy, and related complications

The Korean Association for the Study of the Liver (KASL)

Keywords: Cirrhosis; Varices; Hepatic encephalopathy; Guideline

PREAMBLE

Background and aims

Patients with decompensated cirrhosis with complications have a very poor prognosis and require careful management. Varices are common complications in patients with cirrhosis. Although the prognosis of variceal bleeding has improved with recent advances in diagnosis and treatment, the mortality rate remains 12–22%. Hepatic encephalopathy (HE) is known to occur in 10–14% of patients with cirrhosis and 16–21% of patients with decompensated cirrhosis. More than 20% of cirrhotic patients who visit emergency rooms in Korea present with HE. Therefore, cirrhosis is a serious disease in Korea and requires specific Korean guidelines for diagnosis, treatment, and prevention. In 2005, the Korean Association for the Study of the Liver (KASL) enacted a clinical practice guideline (CPG) for the treatment of cirrhosis complications including ascites, hepatorenal syndrome, varices, and HE. In 2011, the guidelines for the treatment of cirrhosis were revised to integrate antifibrotic treatment and update the diagnosis and treatment advice for variceal bleeding, cirrhotic ascites, and HE. In 2017, the CPG for liver cirrhosis was revised for ascites and related complications. At this time, KASL is revising the CPG for liver cirrhosis to address varices and HE following ascites and related complications. To date, many studies have addressed the prevention and treatment of gastroesophageal variceal bleeding and HE, and many guidelines have been based on those studies, but most of them contain foreign data that are difficult to apply to Korean

Abbreviations:
ANT, animal naming test; BCAAs, branched-chain amino acids; BRTO, balloon-occluded retrograde transvenous obliteration; cACLD, compensated advanced chronic liver disease; CFF, critical flicker frequency; CHE, covert hepatic encephalopathy; CI, confidence interval; CLDQ, Chronic Liver Disease Questionnaire; CPG, clinical practice guideline; CT, computed tomography; DST, digit span test; EEG, electroencephalography; EIS, endoscopic injection sclerotherapy; EVL, endoscopic varical ligation; EVS, esophageal varices; GOV, gastroesophageal varices; GRADE, Grading of Recommendations, Assessment, Development, and Evaluation; HE, hepatic encephalopathy; HR, hazard ratio; HRQoL, health-related quality of life; HVPG, hepatic venous pressure gradient; ICT, inhibitory control test; IL, interleukin; IGV, isolated gastric varices; ISHEN, International Society for Hepatic Encephalopathy and Nitrogen Metabolism; ISMN, isosorbide-5-mononitrate; KASL, the Korean Association for the Study of the Liver; KPPT, Korean paper and pencil test; LDQoL, Liver Disease Quality of Life; LDSI, Liver Disease Symptom Index; LOLA, L-ornithine-L-aspartate; MCS, mental component summary; MHE, minimal hepatic encephalopathy; MRI, magnetic resonance imaging; NCT, number connection test; NHP, Nottingham Health Profile; NSBBs, nonselective beta-blockers; OHE, overt hepatic encephalopathy; OR, odds ratio; PARTO, vascular plug-assisted retrograde transvenous obliteration; PC5, physical component summary; PEG, polyethylene glycol; PHES, psychometric hepatic encephalopathy score; PPI, proton pump inhibitor; PRBC, packed red blood cell; RCTs, randomized controlled trials; RR, relative risk; SDMT, symbol digit modality test; SF-36, Medical Outcomes Study Short Form-36; SF-LDQOL, Short Form Liver Disease Quality of Life; SIP, Sickness Impact Profile; SVR, sustained virologic response; TIPS, transjugular intrahepatic portosystemic shunt

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Editor: Yoon Jun Kim, Seoul National University College of Medicine, Korea

Received : Oct. 22, 2019 / Accepted : Oct. 23, 2019

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clinical practice. Therefore, these revised guidelines for the treatment of varices and HE are offered for Korean practice to reflect the latest research results and extensive discussions within the revision committee. This guideline contains the opinions of experts and is intended to be a practical reference for the care of patients with varices and HE; it is not an absolute standard of care. The best choices for each patient’s care vary from case to case, and the judgment of the doctor in charge is important. As medical evidence and new findings accumulate in the future, these guidelines will require ongoing supplementation and revision. This guideline may not be modified or altered without permission.

**Target population**

This guideline discusses patients with varices, HE, and related complications (esophageal varices [EVs] and bleeding, gastric varices and bleeding, portal hypertensive gastropathy, covert and overt HE) caused by liver cirrhosis. It is intended for clinicians and other medical personnel who are in charge of diagnosing and treating patients with liver cirrhosis. This guideline is also intended to provide practical clinical and educational information and directions for resident physicians and fellows in training, practitioners, and their trainers and supervisors.

**Development, funding, and revision process**

Comprising 14 hepatologists, the Clinical Practice Guideline Committee for Liver Cirrhosis: Varices, HE, and related complications (the Committee) was organized by the KASL Board of Executives. Funding for the revisions was provided by KASL. Each committee member collected and analyzed source data in his or her own field, and the members then wrote the manuscript together.

**Literature review**

The Committee selected keywords and questions using PICO (Patient/Problem, Intervention, Comparison, Outcome) assessments and systematically collected and reviewed international and domestic literature available in PubMed, MEDLINE, KoreaMed, the Korean Medical Database, and other databases. In addition to published articles, abstracts of important meetings published before January 2019 were evaluated.

**Levels of evidence and grades of recommendation**

The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system (Table 1) was applied to grade the evidence and recommendations. The levels of evidence are based on the possibility of change in the estimate of clinical effect by further research and are described as high (A), moderate (B), or low (C). The recommendations are also classified as strong (1) or weak (2) by the GRADE system based on the quality of evidence, the balance between the desirable and undesirable effects of an intervention, generalizability, and socioeconomic aspects (including cost and availability). Each recommendation is labeled with the level of relevant evidence (A–C) and corresponding recommendation grade (1, 2) as follows: A1, A2, B1, B2, C1, C2.

**List of key questions**

The Committee selected the following key questions about varices, HE, and related complications to cover in this guideline.

**Key varix-related questions**

1) How should varices be monitored?
2) Who needs monitoring for varices?
3) How can the development and progression of EVs be prevented?
4) Who needs treatment to prevent initial esophageal variceal bleeding?
5) What is the proper management for preventing initial esophageal variceal bleeding?
6) How can acute esophageal variceal bleeding be diagnosed?
7) What is the appropriate pharmacological treatment for acute esophageal variceal bleeding?
8) What is the proper endoscopic treatment for acute esophageal variceal bleeding?
9) What are the options for rescue treatment when endoscopic treatment of acute variceal bleeding fails?
10) What is the primary treatment to prevent EVs from rebleeding?
11) What are the options for rescue treatment when primary treatment to prevent EVs from rebleeding fails?
12) Who needs treatment to prevent gastric variceal bleeding?
13) What is the proper treatment to prevent gastric variceal bleeding?
14) What is the proper treatment of acute gastric variceal bleeding?
15) What is the primary treatment to prevent gastric varices from rebleeding?

16) How should portal hypertensive gastropathy be classified?

17) How should portal hypertensive gastropathy be managed?

**Key HE-related questions**

1) How should HE be diagnosed and classified?
2) How should overt HE be defined and diagnosed?
3) What are the precipitating factors of overt HE?
4) What differential diagnoses should be considered in diagnosing overt HE?
5) Is the measurement of serum ammonia helpful in diagnosing overt HE?
6) Is radiologic image evaluation of the central nervous system helpful in diagnosing overt HE?
7) What neurophysiological or neuropsychological tests are clinically necessary to diagnose overt HE?
8) How should the acute phase of overt HE be treated, and how should recurrence be prevented?
9) Are branched chain amino acids helpful in treating and preventing overt HE?
10) Is L-ornithine-L-aspartate (LOLA) helpful in treating and preventing overt HE?
11) Is proper education helpful in preventing the recurrence of and readmission for HE?
12) How should covert HE be defined and diagnosed?
13) What is the clinical significance of covert HE?
14) How should covert HE be treated?
15) How should the quality of life of HE patients be assessed?
16) Does treating HE improve patient quality of life?

**Review of the manuscript and approval process**

Each manuscript written by members was reviewed and approved through meetings of the Committee. An updated manuscript was reviewed at a meeting of the advisory board and opened to a public hearing attended by KASL members, members of related organizations, and representatives from patient associations. The final manuscript was approved by the KASL Board of Executives.

**Release of the guidelines and plan for updates**

The revised guideline (The KASL Clinical Practice Guidelines for Liver Cirrhosis: Varices, Hepatic Encephalopathy and Related Complications) was released at a KASL meeting on 22 June 2019. The Korean version of the guideline is available on the KASL website (http://www.kasl.org).

**VARICES**

Varices are a frequent complication of liver cirrhosis and a leading cause of mortality in patients with liver cirrhosis. Varices were present in 52.2% of patients who received endoscopy for variceal screening, and the incidence of varices was significantly higher in patients with Child-Pugh class B/C than in those with Child-Pugh class A (35–43% vs. 48–72%). Portal hypertension, which is
the most common complication of liver cirrhosis, is the main determinant in the development of varices. Increased intrahepatic vascular resistance to portal flow leads to the development of portal hypertension, which is aggravated by splanchnic vasodilatation and an increase in portal blood flow caused by hydodynamic circulation.\textsuperscript{3-5} When the portal pressure increases above a threshold, collaterals develop at the site of communication between the portal and systemic circulation, of which varices are the most important. With the aggravation of portal hypertension, the collaterals grow and eventually rupture. Bleeding from varices is a major complication of portal hypertension and a leading cause of mortality in patients with liver cirrhosis. Therefore, preventing variceal development and progression, preventing bleeding from varices, appropriately managing acute bleeding from varices, and preventing variceal rebleeding are critical in patients with liver cirrhosis.

The incidence of varices in cirrhotic patients without varices at baseline is 5–9% at 1 year and 14–17% at 2 years.\textsuperscript{6,7} The main risk factor for variceal development in these patients is a higher hepatic venous pressure gradient (HVPG).\textsuperscript{8} Small EVs often progress to large varices; the incidence of progression from small to large EVs is 12% at 1 year and 25% at 2 years. The independent risk factors of EV progression are alcoholic cirrhosis, decompensated disease, and splenomegaly.\textsuperscript{7} The 1-year incidence of variceal bleeding in patients with cirrhosis and varices without a previous history of bleeding is approximately 12% (5% for small varices and 15% for large varices), and the main risk factors of bleeding are larger varices, the presence of redness over the varices, and decompensated disease.\textsuperscript{8} Although the mortality rate has decreased significantly during the past several decades thanks to improvements in diagnostic and therapeutic modalities,\textsuperscript{9,10} it remains as high as 12–22%.\textsuperscript{11-14} In addition, rebleeding is frequent, up to 60% within 1 year, without appropriate treatment to prevent it.\textsuperscript{15}

\section*{Surveillance of varices}

\subsection*{Endoscopic surveillance of varices}

Given the high prevalence of varices and poor prognosis with variceal bleeding, monitoring varices is important in patients with liver cirrhosis. Therefore, upon first diagnosis with liver cirrhosis, endoscopy should be performed to look for varices and assess the risk of bleeding. Diagnosis of liver cirrhosis is not difficult in patients with decompensated liver cirrhosis accompanied by ascites or variceal bleeding, but a liver biopsy is needed to diagnose patients with compensated cirrhosis who have no clinical symptoms or signs. However, liver biopsy is an invasive procedure with a risk of serious complications.\textsuperscript{16} Furthermore, doubt has been cast on the accuracy of liver biopsy because of the risk of sampling errors\textsuperscript{7,18} and intra- and interobserver variability.\textsuperscript{18,19}

Liver cirrhosis can disappear with appropriate treatment of the underlying liver disease,\textsuperscript{20,21} though portal hypertension can accompany the severe stage of fibrosis (F3).\textsuperscript{22,23} Various practice guidelines recommend surveillance for hepatocellular carcinoma in patients with liver fibrosis, even before the development of cirrhosis.\textsuperscript{24,25} Therefore, the alternative term compensated advanced chronic liver disease (cACLD) has been proposed for patients with severe fibrosis (F3) and compensated liver cirrhosis to better reflect that the spectrum of severe fibrosis and cirrhosis is a continuum in asymptomatic patients and that distinguishing between these two conditions is often clinically impossible.\textsuperscript{26} A liver stiffness value, measured by transient elastography, of <10 kPa can rule out cACLD, and a value between 10 and 15 kPa is suggestive of cACLD but needs further tests for confirmation. A value >15 kPa is highly suggestive of cACLD.\textsuperscript{26} Endoscopic surveillance of all patients with cACLD can cause problems, such as an increase in medical costs due to an increase in unnecessary tests. Therefore, noninvasive screening tests have been proposed for patients with EVs, especially those whose EVs have a high risk of bleeding, to reduce unnecessary endoscopic surveillance. The Baveno VI criteria suggest that endoscopic surveillance can be avoided in cACLD patients with a liver stiffness <20 kPa and a platelet count >150×10^3/L because they are at very low risk for varices that need to be treated.\textsuperscript{26} Augustin et al.\textsuperscript{27} expanded the Baveno VI criteria to say that endoscopic surveillance can be avoided in cACLD patients with liver stiffness <25 kPa and a platelet count >110×10^3/L. However, considering that noninvasive screening for varices that need to be treated is not particularly reliable\textsuperscript{28,29} and endoscopy is more easily accessed in Korea than in Western countries, we do not deem screening by noninvasive test to be useful in Korea.

\subsection*{Surveillance of EVs}

The incidence of EV development in cirrhotic patients without varices is 5–9% at 1 year and 14–17% at 2 years.\textsuperscript{6,7} Small EVs progress to large varices at the rate of 12% after 1 year and 25% after 2 years.\textsuperscript{7} Therefore, endoscopic surveillance should be performed more frequently in patients with small EVs than in those without EVs. In addition, because the type of underlying liver disease (e.g., alcoholic cirrhosis) and liver function (e.g., decompensated cirrhosis) are risk factors for the progression of EVs, they
should be taken into account when determining the surveillance interval. Endoscopic surveillance should be performed at 2–3-year intervals in patients with compensated liver cirrhosis and at 1–2-year intervals in those with decompensated liver cirrhosis.\textsuperscript{30,31}

EVs can be classified as large or small according to their size, with a breakpoint at 5 mm in diameter,\textsuperscript{32} or they can be classified as F1 (linearly dilated, small and straight varices), F2 (beady varices, tortuous and occupying less than one third of the esophageal lumen), or F3 (nodular varices, large and occupying more than one third of the esophageal lumen).\textsuperscript{13} However, because the F2 and F3 classifications are fairly subjective and prophylactic treatment is recommended both for F2 and F3, F1 is usually classified as small, and F2 and F3 are classified together as large.

\textbf{[Recommendations]}

1. In patients diagnosed with liver cirrhosis, screening endoscopy is recommended to determine the presence of varices and assess the risk of bleeding. (A1)
2. In endoscopy, EVs are classified as small (F1) and large (F2 or F3), and the presence of redness should be evaluated. (B1)
3. To identify the development and progression of EVs, endoscopic surveillance should be performed at 2–3-year intervals in patients with compensated liver cirrhosis and at 1–2-year intervals in those with decompensated liver cirrhosis. The frequency of endoscopic surveillance could be modified according to the type and severity of underlying liver disease. (B1)

\textbf{Preventing the formation and progression of EVs}

Appropriate treatment for the underlying liver disease can improve liver fibrosis, which could improve portal hypertension and prevent the development of complications. In patients with hepatitis B virus-related liver cirrhosis, the cirrhosis disappeared from the liver biopsy reports of 74\% after 5 years of treatment with tenfovir disoproxil fumarate,\textsuperscript{20} and in a meta-analysis, hepatic histologic improvement was observed in chronic hepatitis C patients treated with pegylated interferon±ribavirin.\textsuperscript{44} In an earlier study of patients with nonalcoholic fatty liver disease, the degree of weight loss correlated with the degree of histologic improvement.\textsuperscript{15} Furthermore, the incidence of EVs was significantly lower in patients with a sustained virologic response (SVR) to pegylated interferon±ribavirin treatment than in those without an SVR.\textsuperscript{36-38}

In a recent study, portal pressure was significantly lower in patients with an SVR to direct-acting agents than in those without an SVR in patients with hepatitis C virus-related liver cirrhosis.\textsuperscript{39}

Because the development of GEVs is a direct consequence of portal hypertension, reducing the portal pressure through the use of nonselective beta-blockers (NSBBs) from the early stage of liver cirrhosis could theoretically ameliorate the formation of GEVs. However, a placebo-controlled study to determine whether NSBBs could prevent the formation of varices in 213 patients with cirrhosis and portal hypertension without GEVs, the incidence of varices or bleeding from varices did not differ between timolol group and the placebo group (39\% vs. 40\%, \(P=0.89\)), and serious adverse events developed more frequently in the timolol group than the placebo group (18\% vs. 6\%, \(P=0.006\)).\textsuperscript{6} Therefore, the use of NSBBs to prevent the formation of varices is not recommended.

Several studies have evaluated whether NSBBs can prevent or delay the growth of small varices, and the results conflict. One study found a significant reduction in the rate of progression to large EVs in the nadolol group compared with the placebo group in patients with cirrhosis and small EVs (7\% vs. 31\% at 2 years, 20\% vs. 51\% at 5 years; \(P<0.001\)),\textsuperscript{40} but another study showed that propranolol offered no benefit for the prevention of progression to large varices (23\% in the propranolol group vs. 19\% in the placebo group, \(P=0.786\), even though the reduction in portal pressure was significantly greater in the propranolol group.\textsuperscript{41} A recent meta-analysis suggests that NSBBs are not effective in preventing the progression from small to large varices.\textsuperscript{42} Another study found that the incidence of progression to large varices across 24 months was significantly lower in the carvedilol group than the placebo group (20.6\% vs. 38.6\%, \(P=0.04\)), leading those researchers to suggest that carvedilol is a safe and effective way to delay the progression of small to large EVs in patients with cirrhosis.\textsuperscript{43}

Carvedilol reduces portal pressure by means of an anti-a1-mediated decrease in intrahepatic resistance and splanchnic vasoconstriction. Because intrahepatic vasoconstriction is the main pathologic mechanism in the development of portal hypertension during early-stage liver cirrhosis, it could be more effective than other medications in preventing the progression of varices in patients with early-stage cirrhosis.\textsuperscript{44} However, further studies are needed to confirm the effects of carvedilol.
[Recommendations]
1. Appropriate treatment for the underlying liver disease is recommended to prevent the formation of EVs. (A1)
2. NSBBs (propranolol and nadolol) are not recommended to prevent the formation of EVs in cirrhotic patients without EVs. (A1)
3. In patients with small EVs that are not red, NSBBs (propranolol and nadolol) or carvedilol could be considered to prevent the progression of EVs. (B2)

Prevention of first variceal bleeding in patients with EVs

In patients with liver cirrhosis and EVs, variceal bleeding occurs at a yearly rate of 5–15% of cases. Active prevention of the first variceal bleeding is indicated in patients at a high risk of bleeding, such as patients with large varices (F2, F3), decompensated cirrhosis, or varices with red color signs on endoscopy.8,45

Prevention of first variceal bleeding in patients with small EVs

In cirrhotic patients with small EVs, the risk of bleeding is low (3% at 2 years and 8% at 4 years) and remains low in patients whose varices remain small at the follow-up endoscopy, though it increases significantly when the varices become large. An increase in Child-Pugh score during follow-up appears to be a significant predictor of enlarged varices and thus an increase in bleeding risk.46 The prevention of first bleeding in patients with small EVs depends on their risk of bleeding. Patients with small varices with red color signs on endoscopy or decompensated cirrhosis have an increased risk of bleeding and should consider using NSBBs.26,47

Prevention of first variceal bleeding in patients with large EVs

NSBBs and EVL

Meta-analyses of randomized controlled trials (RCTs) have shown that the use of NSBBs can prevent first variceal bleeding in cirrhotic patients with large EVs.48,49 A study comparing NSBBs and EVL as primary prophylaxis in patients with high-risk EVs found no significant difference between them in bleeding rates (relative risk [RR], 0.86; 95% confidence interval [CI], 0.55–1.35).50 A meta-analysis of RCTs evaluating the efficacy of EVL and pharmacological therapy in preventing first EV bleeding in patients with cirrhosis also found no significant difference in the rate of variceal bleeding between the two groups.51 Another meta-analysis found that EVL significantly reduced the rate of first variceal bleeding and severe adverse events than NSBBs in patients with large EVs.52 Thus, in most studies, the efficacy of EVL in preventing first variceal bleeding was similar to that of NSBBs, and in some studies, the efficacy of EVL was superior to NSBBs. Therefore, either NSBBs or EVL is recommended for the prevention of first variceal bleeding in patients with large EVs. The choice of treatment should be based on clinician expertise and patient preference, characteristics, contraindications, and adverse events.26,47

Carvedilol

Carvedilol is known to be more effective in reducing portal pressure than propranolol.53-55 In a multicenter RCT comparing the efficacy of carvedilol and EVL in preventing first variceal bleeding in cirrhotic patients with large EVs, carvedilol had lower rates of first variceal bleeding (10% vs. 23%, P=0.04), but there was no significant difference in overall mortality or bleeding-related mortality during follow up.56 In another RCT comparing the efficacy of carvedilol and EVL for primary prophylaxis of EV bleeding, the carvedilol and EVL groups had comparable variceal bleeding rates (8.5% vs. 6.9%, P=0.61).57 In a study assessing the efficacy of carvedilol, propranolol, and EVL for the primary prevention of variceal bleeding in patients with large varices, no significant differences among the groups were found in the risk of bleeding (15.4% vs. 10.8% vs. 10.2%, P=0.071), but the incidence of adverse events was the highest in the propranolol group.58 In studies comparing the efficacy of carvedilol, NSBBs, and EVL for the primary prevention of EV bleeding, carvedilol was similar to NSBBs and EVL or superior to EVL. Therefore, carvedilol can also be used to prevent first variceal bleeding in patients with high-risk EVs.

Combination therapy of EVL and NSBBs

The combination of EVL and NSBBs for the primary prophylaxis of variceal bleeding could have a synergistic effect from the direct eradication of varices by EVL and the reduction of portal pressure by NSBBs. Several studies have compared the efficacy of combination therapy with that of monotherapy based on that hypothesis. In RCTs comparing EVL plus propranolol with EVL alone for preventing first variceal bleeding in patients with high-risk EVs, the combination therapy did not show any difference from EVL alone in first bleed occurrence or mortality during follow up. However, the recurrence of varices was lower in the combination
The disadvantages of NSBBs include dizziness, fatigue, general weakness, dyspnea, headache, hypotension, bradycardia, and erectile dysfunction. Discontinuing NSBBs can increase the risk of variceal bleeding and mortality. Thus, treatment with NSBBs should be continued indefinitely. In patients with contraindications or discontinuation due to severe side effects or poor compliance with NSBBs, EVL is recommended.

In patients with end-stage liver disease, such as refractory ascites or spontaneous bacterial peritonitis, the administration of NSBBs has not yet been established. In cirrhotic patients with refractory ascites, the use of NSBBs can lower arterial pressure, decrease survival time, and increase the risk of paracentesis-induced circulatory dysfunction. In addition, among patients with cirrhosis and spontaneous bacterial peritonitis, NSBBs increase the risk of hepatorenal syndrome and acute kidney injury and reduce survival time. However, other studies have reported that the use of NSBBs increased or did not affect survival time in cirrhotic patients with refractory ascites. Another study found that treatment with low-dose propranolol (80 mg/day) increased survival time in patients with spontaneous bacterial peritonitis. The role of NSBBs in patients with refractory ascites or spontaneous bacterial peritonitis thus remains uncertain, and clinicians must carefully consider the risks and benefits when deciding whether to administer them. If NSBBs are administered, thorough monitoring of blood pressure and renal function is necessary, and dose reduction or discontinuation should be considered in patients who develop low blood pressure or impaired renal function. Discontinuation of NSBBs can increase the risk of EV bleeding; thus, if NSBBs are stopped, EVL should be considered.

Carvedilol
Adjusting the dose of carvedilol is easier than adjusting the dose of NSBBs because it is not guided by heart rate. Carvedilol is started at 6.25 mg once a day (or 3.125 mg twice a day), and after 3 days increased to 6.25 mg twice a day. The maximum dose of carvedilol is 25 mg daily in patients without ascites and 50 mg daily in patients with ascites. Systolic blood pressure should not decrease <90 mmHg.

The advantages of NSBBs include low cost, ease of administration, and not requiring follow-up endoscopies. Propranolol is started at 20–40 mg twice a day and adjusted every 2–3 days until the treatment goal is achieved. The maximum dose is 160 mg daily in patients without ascites and 80 mg daily in patients with ascites. Systolic blood pressure should not decrease <90 mmHg.

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**EVL**

The advantages of EVL are that it can be performed in the same session as screening endoscopy, and it has few contraindications. The disadvantages of EVL are the side effects associated with sedation and the risk of causing dysphagia, esophageal ulcerations, strictures, and bleeding. Although the incidence of side effects is higher with NSBBs, severe side effects, such as ulcer bleeding at the ligation site, are more likely to be associated with EVL. 47 Some studies have reported that proton pump inhibitors (PPIs) significantly reduce the size of post-EVL ulcers or the rate of post-EVL ulcer bleeding. 76-78 In cirrhotic patients, the long-term use of PPIs can increase the risk of spontaneous bacterial peritonitis and HE, so PPIs should be used with caution. 79-81 Meanwhile, because EVL is a local therapy that does not act on the pathophysiology of portal hypertension, not only is it unable to prevent complications other than variceal bleeding, but it also requires follow-up endoscopies to assess variceal recurrence, even after variceal eradication, 47 defined as a case in which varices are not seen or become too small to be ligated. Repeat EVL can be performed at intervals of 2–8 weeks until variceal eradication is achieved. Follow-up endoscopies should be performed 1–6 months after variceal eradication and every 6–12 months thereafter. 47,50,82

**[Recommendations]**

1. In cirrhotic patients with small EVs that have a high risk of bleeding (decompensated cirrhosis or red color signs on endoscopy), the use of a NSBBs (propranolol or nadolol) should be considered to prevent first variceal bleeding. (B1) NSBBs are adjusted every 2–3 days until the resting heart rate reaches 55–60 beats per minute.
2. In cirrhotic patients with large EVs, the use of a NSBBs (propranolol or nadolol), carvedilol, or EVL is recommended to prevent first variceal bleeding. (A1) A combination of NSBBs and EVL can also be considered. (B2)

**Diagnosis and management of acute esophageal variceal bleeding**

**Diagnosis of acute esophageal variceal bleeding**

In patients with upper gastrointestinal bleeding, variceal bleeding caused by portal hypertension can be suspected if the patients show jaundice, ascites, HE, splenomegaly, collateral circulation of the abdominal vessels, lower extremity edema, or spider angiommas. A definite diagnosis can be established by endoscopic examination. If blood clots or white nipples appear on the surface of the varices, or if blood is found in the stomach without a potential bleeding focus other than EVs, acute EV bleeding can be diagnosed. 45

**General management of acute esophageal variceal bleeding**

Acute EV bleeding is a medical emergency requiring intensive care. It is essential to protect the circulatory and respiratory status of the patient regardless of the cause of bleeding. Volume resuscitation via adequate fluid therapy and a packed red blood cell (PRBC) transfusion should be initiated to restore and maintain hemodynamic stability. A recent RCT showed that bleeding-related mortality (5% vs. 9%, P=0.02) and the incidence of serious adverse events (12% vs. 18%, P=0.01) were significantly decreased in the "restrictive" PRBC transfusion group (initiating PRBC transfusion at a hemoglobin threshold of 7 g/dL and maintaining it at 7–9 g/dL) compared with the "liberal" PRBC transfusion group. 83 Improved survival in the restrictive transfusion group might be associated with lower rates of hemostasis failure and serious adverse events. In patients with acute EV bleeding, adequate fluid therapy/PRBC transfusion should be performed while considering age, cardiovascular disease, presence or absence of ongoing bleeding, and hemodynamic status. Excessive fluid therapy/PRBC transfusion may increase the portal pressure and aggravate bleeding from the varices, so that should be taken into account. 44 Regarding correction of coagulopathy, clinical studies of recombinant factor VIIa have not shown a clear benefit, and therefore the routine use of fresh frozen plasma or recombinant factor VIIa is not recommended. 85,86 Although the efficacy of platelet transfusion in patients with acute EV bleeding has not been proven because of a lack of clinical studies, it can be considered in patients with severe thrombocytopenia.

**Pharmacological treatment of acute esophageal variceal bleeding**

Cirrhotic patients presenting with acute gastrointestinal bleeding have a high risk of developing bacterial infections, therefore initiation of prophylactic antibiotic treatment at the time of admission is necessary. Meta-analyses of RCTs have shown that the use of antibiotic prophylaxis reduces the risk of infections, recurrent bleeding, and bleeding-related death. 87,88 A recent meta-analysis
demonstrated that prophylactic antibiotic treatment was associated with a decrease in bleeding-related mortality (RR, 0.79; 95% CI, 0.63–0.98), mortality from bacterial infections (RR, 0.43; 95% CI, 0.19–0.97), development of bacterial infections (RR, 0.35; 95% CI, 0.26–0.47), and rebleeding (RR, 0.53; 95% CI, 0.38–0.74). However, another recent retrospective study questioned the usefulness of the routine antibiotic prophylaxis in cirrhotic patients experiencing acute variceal bleeding because of a very low incidence of bacterial infections (2%) and mortality (0.4%) in Child-Pugh class A patients with acute variceal bleeding, even in the absence of prophylactic antibiotic treatment. No prospective study has evaluated the usefulness of antibiotic prophylaxis, and therefore the routine use of prophylactic antibiotics is recommended for all cirrhotic patients presenting with variceal bleeding, regardless of their Child-Pugh class. In a previous RCT comparing intravenous ceftriaxone (1 g every 24 hours) and oral norfloxacin (400 mg every 12 hours) for the prophylaxis of bacterial infection in cirrhotic patients with gastrointestinal bleeding, the incidence of proven or possible infections (11% vs. 33%, P = 0.003), proven infections (11% vs. 26%, P = 0.03), and spontaneous bacterial peritonitis or bacteremia (2% vs. 12%, P = 0.03) was significantly lower in the ceftriaxone group. However, controversy remains about whether those results are applicable to general cirrhotic patients because that was a study conducted in Spain among patients with advanced cirrhosis, and most of the Gram-negative bacilli detected in the patients receiving oral norfloxacin were norfloxacin-resistant strains. Therefore, it is necessary to select appropriate antibiotics based on local antimicrobial susceptibility patterns. Generally, short-term (maximum 7 days) antibiotic prophylaxis with intravenous ceftriaxone (1 g every 24 hours) is recommended in patients with acute variceal bleeding.

Vasoactive agents, such as vasopressin, terlipressin, somatostatin, and octreotide, are effective in supporting hemostasis in patients with acute variceal bleeding by decreasing portal pressure. In a meta-analysis, the use of vasoactive agents in patients with acute variceal bleeding was significantly associated with a reduction in 7-day mortality (RR, 0.74; 95% CI, 0.57–0.95) and an increase in the hemostasis rate (RR, 1.21; 95% CI, 1.13–1.30). In patients with suspected variceal bleeding, vasoactive agents should be initiated as soon as possible, together with prophylactic antibiotics, before the diagnostic endoscopy. Vasopressin reduces portal pressure by inducing systemic and splanchnic vasoconstriction, but it is not now recommended for patients with acute variceal bleeding because of the significant side effects, such as an increase in peripheral vascular resistance and reduction in cardiac output and coronary blood flow. Although terlipressin, a synthetic analogue of vasopressin, is the only drug proven to reduce bleeding-related mortality (RR, 0.66; 95% CI, 0.49–0.88), its side effects, such as hyponatremia and myocardial ischemia due to coronary artery vasoconstriction, should be considered. A recent meta-analysis and a Korean multicenter RCT comparing three vasoactive agents (terlipressin, somatostatin, and octreotide) found no significant differences among them regarding the hemostasis rate and survival time. In patients with acute variceal bleeding, it is recommended that one of the vasoactive agents should be started as soon as possible (Table 2) and continued for 3–5 days.

### Table 2. Vasoactive agents used in the management of acute variceal bleeding

<table>
<thead>
<tr>
<th>Type</th>
<th>Initial dose</th>
<th>Maintenance dose</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terlipressin</td>
<td>2 mg intravenously</td>
<td>1–2 mg intravenously every 4–6 hours</td>
<td>Hyponatremia, myocardial ischemia, abdominal pain, diarrhea</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>250 μg intravenously</td>
<td>250 μg/hr intravenously</td>
<td>Nausea/vomiting, abdominal pain, headache, hyperglycemia</td>
</tr>
<tr>
<td>Octreotide</td>
<td>50 μg intravenously</td>
<td>50 μg/hr intravenously</td>
<td>Nausea/vomiting, abdominal pain, headache, hyperglycemia</td>
</tr>
</tbody>
</table>

Endoscopic treatment of acute esophageal variceal bleeding

If acute variceal bleeding is suspected, endoscopy should be performed as soon as possible to confirm the hemorrhagic focus and hemostasis. Endoscopic hemostasis should be done when acute EV hemorrhage is confirmed by endoscopy. EVL is the endoscopic treatment of choice for patients with acute bleeding from EVs. Endoscopic injection sclerotherapy (EIS) is no longer recommended as standard treatment for acute EV bleeding because of its higher incidence of treatment failure, bleeding-related mortality, and adverse events compared with EVL. In a meta-analysis comparing EVL and EIS in patients with acute EV bleeding, bleeding-related mortality did not differ significantly (RR, 0.95; 95% CI, 0.77–1.17), but the risk of rebleeding was reduced (RR, 0.68; 95% CI, 0.57–0.81) and the rate of variceal eradication was increased (RR, 1.06; 95% CI, 1.01–1.12) in patients undergoing EVL compared with EIS. Most practice guidelines recommend endoscopy within 12 hours after presentation with suspected variceal bleeding.
al bleeding, but that recommendation lacks evidence. A previous Taiwanese retrospective study reported that delayed endoscopy (>15 hours after admission) was an independent risk factor of inhospital mortality (odds ratio [OR], 3.67; 95% CI, 1.27–10.39). In addition, a prospective observational study of 101 patients with acute EV bleeding showed that the 6-week rebleeding rate (18.9% vs. 38.9%, P = 0.028) and mortality (27% vs. 52.8%, P = 0.031) were significantly lowered in patients undergoing early endoscopy (<12 hours) compared with those undergoing delayed endoscopy (>12 hours). However, because those studies were performed without randomization, several confounders that can delay the endoscopy, such as hemodynamic instability, might have influenced the results. Therefore, until the results of large RCTs are reported, endoscopy should be performed as soon as possible in patients with suspected acute EV bleeding. However, the specific timing should be determined by the hemodynamic status of individual patients and the experience and medical resources of the institution.

Once endoscopy and EVL have been performed, early placement of a transjugular intrahepatic portosystemic shunt (TIPS) can be considered in carefully selected patients at high risk for rebleeding. Early TIPS placement reduced the rates of treatment failure and bleeding-related mortality in an RCT of patients with a HVPG >20 mmHg and in an RCT of patients with Child-Pugh class C cirrhosis (score of 10–13) or Child-Pugh class B cirrhosis with active bleeding on endoscopy despite intravenous administration of a vasoactive agent. However, because these two trials excluded patients with Child-Pugh class A cirrhosis, Child-Pugh class B cirrhosis without active bleeding during endoscopy, Child-Pugh class C with a score of 14–15, patients >75 years, HCC beyond the Milan criteria, or a creatinine level greater than 3 mg/dL, it should be considered that those study results apply to only a very small portion of patients with acute variceal bleeding. Notably, a recent prospective observational study showed that the 1-year rebleeding risk was significantly decreased (3% vs. 49%, P < 0.001), but 1-year survival did not differ between patients with and without a TIPS (66.8%±9.4% vs. 74.2±7.8%, P = 0.78). Further studies are needed to evaluate the beneficial effect of early TIPS placement.

Recently, the efficacy of applying hemostatic powder via endoscopy within 2 hours of admission was evaluated in 86 randomized patients with acute variceal bleeding. Cirrhotic patients with acute variceal bleeding received standard medical treatment and were randomized to receive either immediate endoscopy with hemostatic powder application within 2 hours of admission followed by early elective endoscopy the next day (that is, within 12–24 hours of admission) for definitive treatment (EVL for EV bleeding or endoscopic variceal obturation [EVO] for gastric variceal bleeding; study group) or early elective endoscopy only (control group). Improved rates of hemostasis and survival time in the study group suggested the therapeutic potential of endoscopic application of hemostatic powder, an easy procedure requiring minimal expertise.

Rescue treatment for patients with hemostasis failure

Failure to control acute EV bleeding is defined as death or the need to change therapy (defined by one of the following criteria) within 5 days of an acute bleeding episode. A prospective observational study to evaluate the efficacy of TIPS in 58 patients who failed to achieve hemostasis after EIS and pharmacological treatment reported that the TIPS achieved control of the bleeding in 52 patients (90%), and 1-year and 3-year survival rates were 51.7% and 40.2%, respectively. Balloon tamponade is still used as a bridge therapy and provides hemostasis in 80–90% of patients, but the rebleeding rate after deflation is as high as approximately 50%. Moreover, because it is associated with a high rate of serious complications, such as esophageal ulceration, esophageal rupture, and aspiration pneumonia, balloon tamponade should not exceed 24 hours. In a small RCT, a self-expandable, esophageal covered metal stent was tested as an alternative to balloon tamponade in patients in whom pharmacological and endoscopic treatment failed to control bleeding. Although survival in the esophageal stent group was not improved compared with the balloon tamponade group, bleeding control was higher (85% vs. 47%, P = 0.037), and serious adverse events were lower (15% vs. 47%, P = 0.077) in the esophageal stent group. This stent can be placed endoscopically without radiological guidance, and it can stay in place for up to 2 weeks. However, because only 28 patients were included in that study, further study is warranted.
Prevention of esophageal variceal rebleeding

Definition of esophageal variceal rebleeding
EV rebleeding is defined as recurrent bleeding after an absence of bleeding for at least 5 days following recovery from acute EV bleeding. An average of 60% of patients with acute EV bleeding experience rebleeding within 1–2 years, and the mortality rate from rebleeding is 33%. Therefore, appropriate treatment to prevent rebleeding is necessary.

Diagnosis of esophageal variceal rebleeding
The diagnosis of EV rebleeding is the same as the diagnosis of acute EV bleeding. Clinically significant rebleeding can be suspected in a patient who has recurrent melena or hematemesis with 1) hospitalization or the need for a transfusion, 2) a decrease in hemoglobin of more than 3 g/dL, or 3) death within 6 weeks.

Prevention of esophageal variceal rebleeding
NSBBs and EVL are the most common methods used to prevent EV rebleeding. NSBBs, which reduce portal pressure, have been reported to be more effective than placebo at preventing rebleeding in several RCTs. The combination of an NSBBs plus ISMN could improve portal pressure reduction, but it could also increase the incidence of side effects such as headache and dizziness. EVL is the endoscopic treatment of choice for the prevention of EV rebleeding. EVL should be repeated every 2–8 weeks until varical eradication is achieved. Periodic endoscopic follow-up is needed to detect the recurrence of varices even after achievement of variceal eradication. Several systematic reviews and meta-analyses comparing EVL alone to NSBBs alone demonstrated no difference in the rebleeding rate, but the overall mortality rate during follow-up was significant higher with EVL alone (RR, 1.25; 95% CI, 1.01–1.55) or not different. In a long-term follow-up study, the rebleeding rate was higher (30% vs. 64%, P=0.001) but the survival time was longer (30% vs. 49%, P=0.013) in patients treated with the combination of an NSBBs plus ISMN.

Several RCTs and meta-analyses comparing the combination of EVL plus NSBBs to EVL alone or NSBBs alone showed that the combination therapy had lower overall rebleeding and variceal rebleeding. Therefore, the combination of EVL plus an NSBBs has been suggested as the primary treatment for preventing EV rebleeding. A recent meta-analysis demonstrated that the rebleeding rate decreased (RR, 0.44; 95% CI, 0.28–0.69) and the mortality rate during follow-up tended to decrease with the combination of EVL plus a NSBBs (RR, 0.58; 95% CI, 0.33–1.03) compared with EVL alone. However, although the overall rebleeding rate tended to decrease (RR, 0.76; 95% CI, 0.58–1.00), the mortality rate during follow-up did not differ between the combination of EVL plus NSBBs and NSBBs alone. These results suggest the importance of NSBBs in preventing EV bleeding.

RCTs comparing carvedilol to EVL (36.4% vs. 35.5%, P=0.857) and carvedilol to the combination of nadolol plus ISMN (51% vs. 43%, P=0.46) did not show any significant difference in rebleeding rate, and the side effects of carvedilol were less than those with the combination of nadolol plus ISMN (1.6% vs. 28.3%, P<0.0001). Therefore, the use of carvedilol to prevent EV rebleeding can be considered, but no studies have compared the combination of EVL plus carvedilol with the combination of EVL plus an NSBBs, which is currently considered to be the primary treatment to prevent rebleeding. Further studies using carvedilol to prevent EV rebleeding are required.

In a meta-analysis of studies about preventing variceal rebleeding by using NSBBs to reduce portal pressure, the risk of variceal rebleeding was significantly reduced (OR, 0.17; 95% CI, 0.09–0.33; P=0.0001) when the HVPG was decreased to the target level (reduction in HVPG of ≥20% or to ≤12 mmHg) compared to treatment.
with the non-responding group. A recent RCT comparing HVPG-based medical therapy with TIPS placement to reduce variceal rebleeding showed lower incidence of rebleeding within 2 years (26% vs. 7%, P=0.002) in the TIPS group, but there was no significant difference in mortality during follow-up between the two groups, and the incidence of HE was lower (8% vs. 18%, P=0.05) in the HVPG-based medical therapy group. Considering that a TIPS is a limited treatment method, HVPG-based medical therapy is a useful way to prevent rebleeding if HVPG measurement is possible. However, because HVPG measurement is invasive, it is not widely practiced in many hospitals.

An RCT comparing TIPS placement with a combination of EVL plus an NSBBs to prevent variceal rebleeding found a lower variceal rebleeding rate in the TIPS group (0% vs. 29%, P=0.001), but the incidence of HE within 1 year in that group was higher (35% vs. 14%, P=0.035). There was no difference in the follow-up mortality rate (32% vs. 26%, P=0.418) between the two groups. Therefore, the use of TIPS is not recommended as a primary treatment for the prevention of variceal rebleeding, and it should instead be considered a rescue therapy for patients with primary treatment failure. In addition, liver transplantation is considered a rescue therapy for patients with recurrent variceal rebleeding because it exhibits good long-term results.

### Recommendations

1. In patients with acute esophageal variceal bleeding, treatment to prevent variceal rebleeding is recommended. (A1)
2. The combination of endoscopic variceal ligation (EVL) plus NSBBs is recommended as the primary treatment for esophageal variceal bleeding. (A1) If the combination treatment is difficult to perform, use of a NSBBs or EVL alone is recommended. (A1)
3. If primary treatment for esophageal variceal rebleeding fails, TIPS placement should be considered as a rescue therapy. (B1)
4. Liver transplantation might be considered in patients with recurrent variceal rebleeding. (B1)

### Definition of gastric varices and prevention of primary bleeding

#### Definition and classification of gastric varices

Gastric varices are enlarged submucosal veins of the stomach that cause critical upper gastrointestinal bleeding. GVs occur in approximately 20% of patients with portal hypertension, and the bleeding rate in 2 years is known to be 25%. The incidence of gastric varices is lower than that of EVs, but their rebleeding rate and mortality rate are higher because they cause severe bleeding.

![Figure 1](https://www.e-cmh.org/)

**Figure 1.** Classification of gastric varices. PV, portal vein; LGV, left gastric vein; SV, splenic vein; GOV, gastroesophageal varices; PGV, posterior gastric vein; SGV, short gastric vein; IGV, isolated gastric varices; GEV, gastric epiploic vein.
Gastric varices are classified as gastroesophageal varices (GOV) or isolated gastric varices (IGV) depending on their location and relation to any EVs (Fig. 1). GOVs are classified by whether they extend along the lesser curvature (GOV1) or the gastric fundus (GOV2). IGV are classified as varices located in the fundus (IGV1) and those in any other region, i.e., stomach or duodenum (IGV2). The incidence of GOV1s is about 74%.

Prevention of primary bleeding of gastric varices

The risk factors for gastric variceal bleeding are location (IGV1>GOV2>GOV1), variceal size, redness, and severe liver dysfunction. To prevent bleeding from GOV1s, follow the guidelines for the prevention of EV bleeding. In a Korean study of 85 patients with GOV1s, the GOV1s also disappeared when EVs were eliminated by EVL (64.7%). For GOV2s and IGV1s, EVO, balloon-occluded retrograde transvenous obliteration (BRTO), and vascular plug-assisted retrograde transvenous obliteration (PARTO) can be considered to prevent bleeding. NSBBs are non-invasive and can be used because they can reduce other side effects in patients with cirrhosis.

One randomized study reported the prevention of first gastric variceal bleeding. It enrolled 89 patients with GOV2s or IGV1s larger than 10 mm. The effects of EVO (cyanoacrylate), an NSBB, and simple observation were compared. For the prevention of gastric variceal bleeding, EVO (10%) was superior to an NSBB (38%) and simple observation (53%). The survival rate of the EVO group (93%) was higher than that of the simple observation group (73%), but it did not differ from that of the NSBB group (83%). In a meta-analysis of patients with a high risk of gastric variceal bleeding, BRTO was effective in preventing gastric variceal bleeding (clinical success rate, 97.3%). In a recent study of 73 patients, PARTO was found to be a safe procedure without serious side effects that effectively prevented gastric variceal bleeding (Fig. 2).

[Recommendations]

1. Primary prevention of bleeding for GOV1s follows the recommendations for EVs. (B1)
2. The group at high risk for bleeding (redness or severe liver dysfunction) from GOV2s or IGV1s can be treated with BRTO, PARTO, or EVO. (B2)

Management of bleeding from gastric varices

Bleeding from gastric varices is less common than from EVs; however, the risks of rebleeding or varix-related death are much

Figure 2. The prevention of initial variceal bleeding. UGI, upper gastrointestinal; EV, esophageal varix; GV, gastric varix; GOV, gastroesophageal varix; IGV, isolated gastric varix; NSBB, non-selective beta blocker; EVL, endoscopic variceal ligation; RTO, retrograde transvenous obliteration; EVO, endoscopic variceal obliteration.
higher in patients bleeding from gastric varices. The gastric varices that bleed are generally large and have high blood flow in the channel, which makes massive bleeding common in patients with large gastric varices.\textsuperscript{143,145,146} Gastric varices exhibit unique characteristics and have a greater variety of sizes, forms, locations, and collateral vessels than EVs. An individualized approach might be needed because few well-controlled clinical trials have tested treatments for gastric variceal bleeding. Until sufficient evidence accumulates, clinicians should seek the best option for each patient based on the patient’s general condition and bleeding patterns and the clinician’s medical resources and expertise.\textsuperscript{145}

**Management of bleeding from gastric varices**

**Endoscopic therapy**

Urgent endoscopic examination, within 12 to 24 hours, is necessary when a patient is suspected to have active bleeding from gastric varices. Endoscopic examination can visualize the bleeding sites and directly enable proper hemostatic treatments.\textsuperscript{14,147}

**EVO**

EVO achieves hemostasis and induces variceal eradication by an intravariceal injection of tissue adhesive agents (cyanoacrylates). Active or recent bleeding from fundic varices (GOV2s, IGV1s) or GOV1s can be managed with EVO. Special care is needed to prevent complications from the adhesive agents, such as ocular injury, damage to endoscopic devices, or the impaction of an injection needle into a varix.\textsuperscript{148} Medical personnel are advised to wear goggles during the procedure. The working channel of a scope can be occluded by adhesive agent that spills during the procedure, so it can be helpful to flush the channel with olive oil in advance. To inject the sticky mixture quickly, a large needle is generally used (21 G or 22 G). The injection site is determined based on the direction of blood flow inside the varix. Because the intravariceal pressure is usually concentrated in the most protruding part of the varix, avoid that site if possible. The injection needle should be long enough to pass through the thick gastric wall (5 mm or longer). 2-N-butyl cyanoacrylate, which is the most commonly used agent in Korea, is used as a 1:1 mixture with lipiodol to delay the polymerization reaction. About 1 mL of mixture is used in each session, and the injection can be repeated until hemostasis is achieved. The initial volume and ratio of the mixture can be adjusted to accommodate the variceal size, intravariceal blood flow, and bleeding pattern (active or stabilized). If the bleeding is severe or the variceal size is large, the volume of the mixture can be increased to 2 mL at a time. As soon as the injection is finished, 1 mL of distilled water or saline should be pushed into the catheter to ensure that the mixture remaining in the catheter is injected into the varix. Then, the needle should be retracted quickly to prevent intravariceal impaction of the needle. The success rate of EVO for hemostasis was 91–97%, and the rebleeding rate was 17–49% in patients with active gastric variceal bleeding.\textsuperscript{149,153} The common complications following EVO are systemic embolism, infection, fever, gastric perforation, gastric ulcer, and peritonitis.\textsuperscript{154}

**EVL**

As with EVs, EVL is frequently performed for GOV1 bleeding. EVL for gastric varices showed an initial hemostasis rate of 80–90% and a rebleeding rate of 14–56% in patients with GOVs.\textsuperscript{140,155-158} However, it should be noted that the depth and size of gastric varices differ from those of EVs. Ligation might not be adequate due to the thick gastric mucosa. Gastric ulcers, where the bands fall off, will expose submucosal varices directly to gastric acid and food materials. This situation could increase the risk of massive bleeding from the ulcers.\textsuperscript{144,155,158,159} In patients with fundal variceal bleeding, the effect or safety of EVL has not been fully explored. In a small randomized trial, EVL showed a significantly higher rebleeding rate than EVO in patients with IGV1 bleeding (83.3% vs. 7.7%, \(P=0.003\)).\textsuperscript{155}

**Radiologic intervention**

Radiologic intervention is one useful hemostatic therapy for the management of bleeding from gastric varices. Sufficient consultation with interventional radiologists is needed in advance. Imaging tests, such as computed tomography (CT), should be performed before the procedure to confirm that the collateral veins are accessible and that no contraindications to the procedure are present.

**TIPS**

TIPS placement is a procedure that robustly decompresses portal hypertension by making a bypass between the hepatic vein and the portal vein. In small non-randomized trials, both TIPS and EVO achieved a hemostasis rate of more than 90%. Complications, such as HE and stent occlusion, and medical costs were higher with the TIPS than with EVO.\textsuperscript{150,154} However, TIPS placement is a useful rescue therapy when initial hemostasis fails.\textsuperscript{162-164} The success rate of TIPS in controlling bleeding as a rescue therapy is 90–100%, with a rebleeding rate of 16–40%.\textsuperscript{162-164} Moreover, since non-covered stents have been replaced by covered
stents, the occlusion and stenosis rates have decreased to 8%.\textsuperscript{167,168} HE can be prevented by decreasing the stent diameter. In a randomized study, the incidence rates of HE within 2 years were 43% and 27% in patients with a conventional stent (10 mm) and those with a smaller one (8 mm), respectively (P=0.03).\textsuperscript{168} TIPS is contraindicated in patients with heart failure or severe pulmonary hypertension because it can abruptly increase preload to the heart. It is difficult to perform the procedure in patients with main portal vein thrombosis. When a cyst, abscess, or mass is blocking the accessible tract in the liver or the infrahepatic bile ducts are markedly dilated, it is difficult to perform TIPS.\textsuperscript{169}

**RTO**

RTO obliterates gastric varices by infusing a sclerosant or embolic agent in a retrograde manner through a gastrorenal shunt. An accessible shunt should be confirmed by CT prior to the procedure. After occluding the shunt with a balloon catheter, a sclerosant, such as ethanolamine oleate or sodium tetradecyl sulfate, is infused into the gastric varices.\textsuperscript{170,171} In a recent, large, retrospective study, the technical success rate of BRTO was 95%.\textsuperscript{172} Another multicenter study, in which 23% of patients had GOV1s, had a technical success rate of 97%.\textsuperscript{173} However, the EVs recurred or became aggravated in 20–41% of patients after the procedure.\textsuperscript{172,173} A recent meta-analysis also showed favorable results. The technical success and major complication rates of BRTO were 96.4% and 2.6%, respectively. The clinical success rate, defined as no recurrence of gastric varices or complete obliteration of varices on subsequent imaging, was 97.3%.\textsuperscript{142}

If a shunt is too large for balloon catheter occlusion, BRTO is not possible. Moreover, BRTO requires that patients retain the balloon catheter for several hours, until the sclerosing agent has hardened in the varices. In rare cases, the balloon can rupture during the procedure, and a systemic embolism of the sclerosing agent can occur. Therefore, a novel intervention, PARTO, was recently developed. PARTO uses a vascular plug with or without coils instead of a balloon and uses a gelatin sponge as the embolic agent.\textsuperscript{143} A multicenter prospective study showed that complete thrombosis of gastric varices and shunts was achieved in 98.6% of patients. No recurrent variceal bleeding or development of HE occurred during follow-up. Moreover, 40% of patients showed improvement in their Child-Pugh scores.\textsuperscript{144} Thus, PARTO is a noteworthy treatment that can replace BRTO in patients with gastric varices and a gastrorenal shunt. However, more data on the long-term efficacy and safety of PARTO are needed.

**Treatment of gastric variceal bleeding**

**General management of gastric variceal bleeding**

In patients with cirrhosis and acute upper gastrointestinal bleeding, a restrictive blood transfusion strategy (with a target range for the post-transfusion hemoglobin level of 7 to 9 g/dL) and antibiotic prophylaxis improved survival.\textsuperscript{83,174} Although patients enrolled in the studies were small, the same transfusion strategy can be recommended for those with gastric variceal bleeding. The beneficial effects of vasoactive agents (terlipressin, octreotide, somatostatin) have not been fully proved in patients with gastric variceal bleeding, either. However, considering their ability to decrease portal hypertension, their use in patients with bleeding from gastric varices can be recommended.\textsuperscript{91,158,175,176}

**Treatment of GOV1 bleeding**

GOV1s, which are an extended type of EV, develop along the lesser curvature and receive blood from the left gastric vein. When EVs are eradicated by endoscopic treatments, the gastric varices also concomitantly disappear in 60–65% of patients.\textsuperscript{134,140} Because of their close relationship in pathophysiology, the management of bleeding from cardiac varices (GOV1s) is similar to that for EV bleeding.\textsuperscript{177} However, it should be noted that sufficient ligations can be difficult for gastric varices because of their large size and deeper location. Furthermore, subsequent post-ligation ulcers might be exposed to gastric acid or food material.\textsuperscript{154,155,158,159} According to small clinical trials and observational studies, EVO produces more favorable outcomes than EVL. The initial hemostasis rates with EVO and EVL in patients with GOV1 bleeding were 85–100% and 80–90%, respectively. The rebleeding rates following EVO and EVL were 3–26% and 14–56%, respectively.\textsuperscript{140,155,158} However, most of those trials were small; the evidence needed to recommend one of these treatments over the other remains insufficient.\textsuperscript{140,155,157,158,178} Therefore, clinicians may choose either EVO or EVL based on their expertise, available medical resources, and the variceal condition (size or extent).

**Treatment of GOV2 or IGV1 bleeding**

GOV2s are a type of gastric varix that extends from EVs toward the fundus. IGV1s are varices localized in the fundus in the absence of EVs.\textsuperscript{134} Both GOV2s and IGV1s are usually called gastric fundic varices. Unlike EVs, fundic varices are supplied with blood from the posterior gastric vein or short gastric vein.\textsuperscript{179,180} Bleeding from the fundus usually occurs in a stage of large varix. Management of fundic variceal bleeding can be difficult because massive
or recurrent bleeding is frequently accompanied. Moreover, collateral shunts or blood circulation around the fundic varices are very diverse. Therefore, it is difficult to apply simple or uniform treatments for fundic variceal bleeding.\textsuperscript{181} Urgent endoscopic examination is always needed in patients with suspicious fundic variceal bleeding in order to direct visualization of bleeding sites and to apply immediate treatments. EVO is one of the most commonly performed in patients with bleeding from fundic varices.\textsuperscript{182} EVO achieved initial hemostasis more often than EVL (OR, 4.44; 95% CI, 1.14–17.3). In particular, the rebleeding rate following EVO was significantly lower than that following EVL in patients with IGVs (OR, 0.06; 95% CI, 0.01–0.58).\textsuperscript{183} TIPS placement and EVO are both effective treatments to control bleeding, with a hemostasis rate of more than 90%. Because of complications such as HE, stent occlusion, and higher cost, TIPS placement over EVO is not recommended as a first-line treatment.\textsuperscript{180,181} However, TIPS placement is an effective rescue therapy when endoscopic therapy fails. The hemostasis rate of TIPS in a rescue setting is 90–100%.\textsuperscript{162,166} BRTO also achieved a high hemostasis rate (more than 90%).\textsuperscript{184,185} However, BRTO showed a significantly lower rebleeding risk (OR, 0.27; 95% CI, 0.09–0.81) and a lower risk of HE (OR, 0.05; 95% CI, 0.02–0.13) than TIPS.\textsuperscript{186} Improvement in liver function was also demonstrated following BRTO.\textsuperscript{187} However, all those results are based on mostly small retrospective studies.

In a small prospective study, BRTO and EVO had similar hemostasis and technical success rates. However, the rebleeding rate was significantly lower in the BRTO group than the EVO group (15.4% vs. 71.4%, P < 0.01).\textsuperscript{188} These results should be interpreted carefully, however, because BRTO was performed only in patients without active bleeding; all the patients with active bleeding were treated with EVO.

In summary, current data suggest that EVO, TIPS, BRTO, or (theoretically) PARTO can be used as the initial treatment for patients bleeding from fundic varices. Because of a lack of evidence, treatments should be chosen based on individual situations in consideration of patients’ safety and the applicability of each therapy in the relevant medical facility.

**Use of PPIs**

Currently, PPIs are used in many patients to prevent ulcer bleeding following endoscopic treatments. However, their effectiveness and duration of treatment have not been fully explored. Long-term use of PPI can increase risk of infection and subsequently cause spontaneous bacterial peritonitis and HE.\textsuperscript{72} However, a recent retrospective study showed that PPI use decreased the rebleeding risk following EVO (OR, 0.554; 95% CI, 0.352–0.873).\textsuperscript{189}

**Rescue therapy in case of endoscopic failure**

A TIPS can be urgently placed when endoscopic treatments fail. The hemostasis rate with rescue TIPS was 90–96% in patients with gastric varices, which is comparable to that with EV bleeding.\textsuperscript{162,163} In a few small studies, BRTO also showed comparable outcomes in patients who failed to achieve initial hemostasis. BRTO can be considered as a rescue therapy when a patient was hemodynamically stabilized and has an accompanying gastrorenal shunt.\textsuperscript{184,186} As a bridging therapy, a balloon tamponade can be applied to control massive bleeding until rescue therapy is ready.\textsuperscript{109}

<table>
<thead>
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<th>[Recommendations]</th>
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<tbody>
<tr>
<td>1. In patients with gastric variceal bleeding, general management, such as prophylactic antibiotics, restrictive transfusion, and vasoactive agents, can be provided as they are for esophageal variceal bleeding. (B1)</td>
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<tr>
<td>2. Gastric varices extending from EVs along the lesser curvature (GOV1s) can be treated with either EVO or EVL, depending on the size and location of the bleeding varix. (B1)</td>
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<td>3. In patients with bleeding from fundic varices (GOV2s, IGV1s), EVO should be considered first. (A1) Retrograde transvenous obliteration (BRTO or PARTO) or TIPS can be used depending on the bleeding status (active or stabilized) and the presence of an accessible shunt. (B1)</td>
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<td>4. A PPI can be used following endoscopic treatments to prevent post-procedure ulcer bleeding. (B2)</td>
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<td>5. Retrograde transvenous obliteration (BRTO or PARTO) or TIPS should be considered as a rescue therapy when endoscopic treatments fail. (B1)</td>
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<td>6. Until a rescue therapy is ready, a balloon tamponade can be applied as a bridging therapy. (B2)</td>
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**Prevention of rebleeding**

GOV1s can be managed in the same way as EVs to prevent rebleeding. The eradication of concurrent EVs with EVL and an NSBB can be used if the EVs are medium to large in diameter. Gastric varices subsequently disappeared in 65% of patients when EVs were controlled.\textsuperscript{140} The rebleeding rate from GOV1s following eradication of EVs was 16–42%.\textsuperscript{150,156} Esophageal EVL can be performed simultaneously with or after treatments for gastric varices. In terms of gastric varices, EVO showed a significantly lower re-
bleeding rate than EVL in patients bleeding from GOV1s (OR, 0.39; 95% CI, 0.16–0.94). However, those studies included only a small number of patients. In a retrospective Korean study, EVO showed beneficial outcomes, with lower 1-year rebleeding rate (3.6% vs. 30.8%, *P*=0.004) and bleeding-related mortality rate (5% vs. 22%, *P*=0.05) than EVL. In a different small study, TIPS placement showed a significantly lower rebleeding rate than EVO (21% vs. 65%, *P*<0.02). However, it is difficult to draw conclusions from that study alone because its rebleeding rate following EVO was relatively higher than previous reports. If an accessible gastrorenal shunt is identified, BRTO or PARTO might be considered. Unfortunately, evidence to support those interventions in patients with GOV1 bleeding is very limited.

In patients bleeding from fundic varices (GOV2s or IGV1s), the only predictor for rebleeding following EVO was variceal size (F3). The use of NSBBs failed to decrease the rebleeding rate. In an RCT, eradication of gastric varices with repeated EVO lowered the rebleeding rate significantly compared with NSBBs (10% vs. 44%, *P*=0.004). There were no differences in rebleeding (54% vs. 47%, *P*=0.609) or bleeding-related mortality (42% vs. 47%, *P*=0.766) between EVO alone and EVO plus an NSBB, respectively. Therefore, use of an NSBB is not recommended to prevent recurrent bleeding from fundic varices. However, NSBBs should be considered if patients have significant portal hypertension or other proven indications, such as large EVs. Clinical trials comparing the rebleeding rates after repeated EVO and TIPS or BRTO are needed.

**Figure 3.** The treatment of acute variceal bleeding and prevention of variceal rebleeding. UGI, upper gastrointestinal; EV, esophageal varix; GOV, gastroesophageal varix; IGV, isolated gastric varix; EVL, endoscopic variceal ligation; EVO, endoscopic variceal obturation; RTO, retrograde transvenous obliteration; TIPS, transjugular intrahepatic portosystemic shunt; NSBB, non-selective beta blocker.
scarce. In a small randomized study of patients with GOV2 bleeding, there was no significant difference in the rebleeding rate between EVO repeated every 4 weeks and TIPS placement (16% vs. 0%, P > 0.05). However, TIPS placement was associated with a higher incidence of complications than EVO. In a meta-analysis, BRTO (7.4%) showed a much lower rebleeding rate than TIPS (22.8%) (OR, 0.27; 95% CI, 0.09–0.81). For GOV2s, treatment of the accompanying EVs can be performed with or after the treatment of fundic varices, according to the guidelines for treating EVs (Fig. 3).

**[Recommendations]**

1. In patients with remnant or recurrent GOV1s following initial treatments, repeated EVO or EVL can be performed to prevent rebleeding. (B2)
2. In patients with remnant or recurrent fundic varices (GOV2s, IGV1s), EVO or RTO (BRTO or PARTO) can be performed. (B2) If there is no accessible shunt or if complications related to severe portal hypertension (recurrent bleeding from EVs, refractory ascites, or hydrothorax) are not controlled, a TIPS can be placed. (B2)

**Other variceal bleeding**

In cirrhosis, variceal bleeding at sites other than the stomach and esophagus is very rare, and there are no established treatment guidelines. The most common locations are the rectum, duodenum, and postoperative stomach. A multi-disciplinary approach involving an endoscopist, interventional radiologists, and surgeons should be used to account for the vascular supply. EVO, BRTO, PARTO, TIPS, the coil inserting method, and the like can all be used.

**Portal hypertensive gastropathy**

**Definition and diagnosis**

Although the incidence of portal hypertensive gastropathy bleeding in cirrhosis is not high, some patients experience poor quality of life due to chronic bleeding and the associated iron-deficiency anemia and repeated transfusions. Portal hypertensive gastropathy is diagnosed when gastric mucosal changes cause a snake-skin appearance or mosaic pattern on endoscopy in patients with portal hypertension. When gastric mucosal changes alone are found, it is diagnosed as a mild form. When red or dark brown viscous changes are found along with changes in the gastric mucosa, it is considered to be severe (Fig. 4). Severe portal hypertensive gastropathy causes more chronic bleeding than the mild form.

Portal hypertensive gastropathy is associated with portal hypertension and causes gastric mucosal changes in the stomach and body, and 30% of patients with gastric antral vascular ectasia

---

**Figure 4.** Classification of portal hypertensive gastropathy.
(watermelon stomach) also have portal hypertension. It is unclear whether portal hypertension is involved in the development of gastric antral vascular ectasia. Gastric antral vascular ectasia causes dilated vessels with fibrin thrombi and fibromuscular hyperplasia of the lamina propria.202

**Treatment of portal hypertensive gastropathy**

In chronic bleeding caused by portal hypertensive gastropathy, the goal of treatment is lowering the portal pressure with NSBBs, vasoconstrictors, or a TIPS.203,204 In cases with active bleeding, endoscopic treatment with argon plasma coagulation can be used. In addition, iron supplementation is recommended.205

**HEPATIC ENCEPHALOPATHY**

HE occurs in more than 10% of all cases of cirrhosis and is a critical complication that seriously reduces the quality of life.206 Because HE can cause serious losses not just for individuals, but also socioeconomically, preventive therapy is of paramount importance. However, because the pathophysiological factors in the development of HE and biomarkers to predict the occurrence of HE have not been sufficiently identified, there are no standardized criteria for diagnosing, classifying, or evaluating the treatment response to HE. It is imperative that those criteria be established in Korea. In particular, quality-of-life assessments and diet and exercise education for patients with HE are clinically important and need to be actively developed.

**Definition of HE**

HE is a neuropsychiatric syndrome caused by hepatic dysfunction that manifests as various neurologic and psychiatric abnormalities.207-209 Clinically, it is classified into overt and covert encephalopathy. Overt HE (OHE) is defined as the occurrence of disorientation, flapping tremor, or asterixis (Table 3). Covert HE (CHE) includes minimal encephalopathy in which cognitive impairment cannot be identified without a cognitive function test and West-Haven criteria grade 1 HE, which means mild cognitive or behavioral change without disorientation.720 The prevalence of HE is reported to be 10–14% of cirrhotic patients and 16–21% of patients with decompensated liver cirrhosis.206,211 In Korea, HE was found in 16–21% of hepatitis B virus–related decompensated liver cirrhosis patients.212 Moreover, 20% of cirrhotic patients admitted to the emergency department were reported to have HE.213

HE is classified according to the underlying liver disease, clinical course, precipitating factors, and severity of neurologic symptoms.214 By underlying liver disease, HE is subdivided into three groups: from acute liver failure, from portosystemic bypass or shunting, and from portal hypertension caused by chronic liver disease. HE caused by portal hypertension is classified as episodic, recurrent (more than two times per year), and persistent HE (no fully recovery from behavioral change). When classified by the precipitating factors, HE is divided into precipitated and spontaneous types. Precipitating factors include gastrointestinal bleed-

### Table 3. Definition and classification of hepatic encephalopathy

<table>
<thead>
<tr>
<th>Classification</th>
<th>Grade</th>
<th>Manifestation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covert</td>
<td>Minimal</td>
<td>No clinical cognitive impairment. Psychometric or neuropsychological alterations can be found in tests exploring psychomotor speed/executive functions or neurophysiological alterations without clinical evidence of mental change</td>
<td>Only psychometric or neurological tests can detect the abnormalities</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Despite being oriented in time and space, the patient appears to have some cognitive/behavioral decay with respect to his or her standard on clinical examination or to the caregivers</td>
<td>Clinical findings usually not reproducible</td>
</tr>
<tr>
<td>Overt</td>
<td>2</td>
<td>Disoriented in time (at least three of the following are wrong: day of the month, day of the week, month, season, or year) plus the other mentioned symptoms</td>
<td>Disorientation and flapping tremor are characteristic. Clinical findings are variable, but reproducible</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Disoriented also in space (at least three of the following are wrongly reported: country, state [or region], city, or place)</td>
<td>Myoclonus, hyperreflexia</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Does not respond even to painful stimuli</td>
<td>Coma</td>
</tr>
</tbody>
</table>
ing, uremia, sedatives, diuretics, protein overload, infection, constipation, dehydration, and electrolyte imbalance. The severity of HE is classified using the West-Haven criteria (Table 3).

**Diagnosis of HE**

**Clinical symptoms**

HE presents with a wide range of clinical patterns, from minimal HE (MHE), in which cognitive impairment cannot be identified without a cognitive function test, to OHE, which is easily detected based solely on symptoms and does not require a cognitive function test. As HE progresses, symptoms such as personality changes, indifference, anxiety, and irritability appear and can reduce sleep quality and quality of life. In some patients, increased muscle tension, hyperreactivity, and the Babinski reflex are present, and they are rarely accompanied by seizures. The flapping tremor, a phenomenon in which hand tremors are caused by incongruity in the tension of various muscles resulting from hyperextension of the wrist as the fingers are spread apart, is a common symptom in the early and middle phases of OHE.

**Severity classification**

The severity of HE is classified using the West-Haven criteria and the Glasgow Coma Scale, with the former used as the basic diagnostic criteria. However, due to their large number of subjective factors, the West-Haven criteria suffer from significant interobserver deviation, which makes it difficult to diagnose the first stage (grade 1) of HE in a clinical setting. Therefore, MHE and stage 1 HE are classified as CHE (Table 3). The International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) defines the onset of disorientation or flapping tremor as the start of OHE. The differential diagnosis for HE requires differentiation from underlying brain diseases, such as cerebral hemorrhage and edema, that can accompany cognitive dysfunction. It should also be differentiated from substance abuse, alcoholism, hyponatremia, and psychiatric illnesses. In chronic alcoholics in particular, it can be difficult to differentiate HE from other alcohol-related neurological diseases. For example, Wernicke’s encephalopathy is marked by eye movement paralysis, gaze-induced nystagmus, and gait disturbances, in addition to memory lapses. Delirium caused by withdrawal from alcohol also needs to be differentiated from HE. Delirium that results from alcohol withdrawal is characterized by an increased heart rate, cold sweats, loud shouting, and a harsh and repetitive tremor. A differential diagnosis is required for acute hyponatremia, hypoglycemia, and metabolic alkalosis because each can present with symptoms similar to those of HE. The differential diagnosis for hyponatremia requires particular caution because its symptoms are very similar to those of HE, and hyponatremia itself can lead to HE. Subdural hematoma can also present with symptoms similar to those of HE and should be carefully differentiated. Cases of subdural hematoma are commonly accompanied by other neurological symptoms, such as hemiplegia. Encephalitis often presents with symptoms such as headache, fever, vomiting, and stiff neck, but a differential diagnosis is required because those symptoms are not always clear and can be accompanied by sleepiness, drowsiness, and unconsciousness. In cases of dementia, the symptoms appear relatively gradually in most cases, whereas alcohol-related dementia often includes violent tendencies caused by frontal lobe damage, as well as the inability to remember recent events.

**Diagnostic tests**

OHE can be diagnosed based solely on clinical symptoms, but other diseases that can cause cognitive dysfunction should still be ruled out. Brain CT and brain magnetic resonance imaging (MRI) are helpful for differentiating neuropsychological abnormalities caused by underlying brain diseases, such as intracranial hemorrhage. Because the risk of cerebral hemorrhage is about five times higher in patients with liver cirrhosis than in healthy people, brain CT or MRI should be performed if a brain lesion is suspected. Brain MRI, in particular, is helpful for diagnosing HE, in which brain edema is associated with nonspecific symptoms such as headache and vomiting, when acute liver failure is suspected. On T1-weighted MRI, an increased signal in the basal ganglia is commonly observed, but those changes lack the sensitivity and specificity required to diagnose HE. If the diagnosis of HE is difficult, neurophysiological or neuropsychological tests can also be performed. In HE, a characteristic, slow triphasic wave is observed during electroencephalography (EEG). This slow triphasic wave is an overall periodic waveform in the bilateral frontal lobes that demonstrates bilateral synchronization and is often accompanied by slow background activity; it is usually seen in phase 2 or 3 HE and disappears in comatose patients. Once a slow triphasic wave has developed, the clinical
outcome is reportedly very poor. In recent studies, the decrease in EEG amplitude in patients with OHE was associated with the severity of HE.

The brainstem auditory-evoked-potential test is sensitive for the diagnosis of CHE. Patients with liver cirrhosis accompanied by CHE exhibit conduction time delays (I–V latency) from the auditory nerve to the midbrain and conduction time delays (III–V latency) from the pontine to the midbrain on the brain auditory-evoked-potential test. It is also known that the risk of developing OHE is increased when abnormal findings are observed on the brain auditory-evoked-potentials test. However, in a study using the cortical auditory-evoked-potential test, the N200 latency was increased in patients with HE. Therefore, the diagnosis of HE cannot be made using EEG alone; further research is required to determine the usefulness of evoked-potential EEG in the diagnosis and prognosis of HE.

Serum ammonia

The venous blood ammonia level is not proportional to the degree of HE and has no association with its prognosis. The metabolism of ammonia is greatly influenced by various organs, such as the kidneys, muscles, brain, and bowel, as well as the liver. However, repeated measurements of ammonia concentrations can help to determine a treatment’s effects. If patients with suspected OHE have normal ammonia concentrations, attention should be paid to the differential diagnosis to look for other diseases. There are various methods of measuring ammonia concentrations, such as those involving the venous or arterial blood or plasma. Because the normal range varies depending on the specific measurement method, a suitable reference value should be used. Although the partial pressure of ammonia gas in arterial blood is thought to be closely related to both the neurophysiological test results and the ammonia concentration in the blood-brain barrier in patients with HE, additional studies are needed to determine the clinical usefulness of that value. Regarding other serum markers, some studies have reported increases in the serum S100β concentration that were proportional to the cognitive function test results in HE patients.

[Recommendations]

1. To confirm the diagnosis of OHE, other diseases that can cause cognitive impairment must first be ruled out, and the diagnosis must be made based on clinical symptoms. (A1)
2. HE is classified as either OHE, which can be diagnosed using only symptoms, or CHE, which requires a cognitive function test. (B1)
3. In patients with suspected HE, imaging tests, including a brain MRI or a neurophysiological test, can be performed to rule out other diseases that can cause cognitive impairment. (B2)
4. Venous blood ammonia levels are not proportional to the degree of HE and are not associated with its prognosis. (A1) However, if patients with suspected HE show normal ammonia concentrations, differentiation from other diseases is required. (B1)

Management of overt HE

The goals of treatment

The goals of treatment are as follows: 1) prevention of secondary damage caused by decreased consciousness and normalization of the patient’s state of consciousness, 2) elimination of social and economic restrictions by preventing recurrence, and 3) improvement of patient prognosis and quality of life. Therefore, appropriate supportive care should be provided to prevent secondary damage (e.g., fall-related injuries or aspiration pneumonia) from an altered consciousness. Furthermore, the precipitating factors should be identified and managed appropriately as soon as possible, and treatments should be initiated using medications that can decrease or eliminate the production of ammonia, the major pathogenic material.

Identification of precipitating factors and management

The precipitating factor can be identified in 80–90% of patients with HE. In many cases, HE can be improved simply by eliminating the precipitating factor; therefore, identifying and promptly managing the precipitating factors is required. The currently known precipitating factors of HE and the corresponding diagnostic tests and treatments are shown in Table 4. According to reports from patients in the Republic of Korea, gastrointestinal bleeding, infection, dehydration by paracentesis, and constipation were the major precipitating factors.

Management of overt HE

Non-absorbable disaccharides

The primary treatment for HE is nonabsorbable disaccharides
such as lactulose ($\beta$-galactosido-fructose) or lactitol ($\beta$-galacto-side sorbitol), which lead to recovery in 70–90% of HE patients.\textsuperscript{217} Therapeutic mechanisms involve the reduction of intestinal pH by the production of acetic and lactic acids (via bacterial degradation of lactulose). Another potential mechanism is the ability of the nonabsorbable disaccharides to increase the count of lactobacillus, which do not produce ammonia. Furthermore, nonabsorbable disaccharides convert ammonia to ammonium, rendering it less absorbable, and they also produce an osmotic laxative effect that flushes the ammonia out.\textsuperscript{93,217} Based on many clinical studies and their low cost, nonabsorbable disaccharides are recommended as an initial therapeutic option.\textsuperscript{209,240} Uribe et al.\textsuperscript{243} found that a 20% lactitol enema had higher efficacy in improving symptoms than a tap water enema (100% vs. 20%, $P=0.0037$) and that the overall response rate to nonabsorbable disaccharides–based therapy was 82.5%. According to a systematic review and meta-analysis,\textsuperscript{244} lactulose or lactitol was more effective in improving symptoms than placebo, with a RR of 0.62 (95% CI, 0.46–0.84), and that the overall response rate to nonabsorbable disaccharides–based therapy was 82.5%. According to a systematic review and meta-analysis,\textsuperscript{244} lactulose or lactitol was more effective in improving symptoms than placebo, with a RR of 0.62 (95% CI, 0.46–0.84). This finding was reproduced in another recent study,\textsuperscript{245} which found an RR of 0.63 (95% CI, 0.53–0.74). When overt HE occurs, 30–45 mL of lactulose (20–30 g) every 1–2 hours should be administered orally until the patient is having at least 2 bowel movements a day. An equivalent daily dose of lactitol is 67–100 g.\textsuperscript{246} Thereafter, the dose should be titrated to achieve two to three soft stools per day. If patients are unable to take medications orally, administration via nasogastric tube might be tried. If patients have severe HE (West-Haven criteria of grade 3 or more) or are unable to take medications orally or via nasogastric tube, an enema of 300 mL lactulose and 700 mL water can be performed 3–4 times per day until clinical improvement is noted.\textsuperscript{240,243,247,248} In this situation, the enema solution should be retained in the intestine for at least 30 minutes.\textsuperscript{93}

**Non-absorbable antibiotics**

Rifaximin, a rifamycin derivative, maintains high concentration levels in the intestine because it is not absorbed, and it remains in an active form until it is excreted.

It inhibits bacterial RNA synthesis by binding to bacterial DNA-dependent RNA polymerase, and it has broad antimicrobial activity against aerobic and anaerobic gram-positive and gram-negative bacteria.\textsuperscript{93} So far, several studies have shown that rifaximin has a positive effect in managing HE.\textsuperscript{242,249-251} Several RCTs with small sample sizes have assessed the effect of rifaximin as a first-line regimen for OHE. A meta-analysis of those RCTs found that rifaximin had a therapeutic effect similar to that of lactulose or lactitol.\textsuperscript{249,251-254} Furthermore, in a recent RCT, patients treated with a combination of rifaximin and lactulose showed a better recovery from HE within 10 days (76% vs. 44%, $P=0.004$) and shorter hospital stays (5.8 vs. 8.2 days, $P=0.001$) than those treated with lactulose alone.\textsuperscript{255} The maximum dose is 1,200 mg/day, which might limit its use in cases of severe HE (West-Haven criteria of grade 3 or more) because of the need for oral ad-

### Table 4. Diagnostic tests to identify the precipitating factors of hepatic encephalopathy and their treatments

<table>
<thead>
<tr>
<th>Precipitating factor</th>
<th>Diagnostic tests</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal bleeding</td>
<td>Endoscopy, complete blood count, digital rectal examination, stool blood test</td>
<td>Transfusion, treatment through endoscopy or interventional radiology, vasoactive drugs</td>
</tr>
<tr>
<td>Infection</td>
<td>Complete blood count (white blood cell differential count), C-reactive protein, chest X-ray, urinalysis and urine culture, blood culture, diagnostic paracentesis</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>Constipation</td>
<td>History-taking, abdominal x-ray</td>
<td>Enema or laxatives</td>
</tr>
<tr>
<td>Excessive protein intake</td>
<td>History-taking</td>
<td>Limiting protein intake</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Skin elasticity, blood pressure, pulse rate</td>
<td>Stop or reduce diuretics, fluid therapy (e.g., intravenous albumin infusion)</td>
</tr>
<tr>
<td>Renal dysfunction</td>
<td>Serum urea nitrogen, serum creatinine, serum cystatin C, serum electrolyte</td>
<td>Stop or reduce diuretics, fluid therapy (e.g., intravenous albumin infusion)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>Serum sodium concentration</td>
<td>Stop or reduce diuretics, fluid restriction</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>Serum potassium concentration</td>
<td>Stop or reduce diuretics</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>History-taking</td>
<td>Stop benzodiazepine, flumazenil</td>
</tr>
<tr>
<td>Opioids</td>
<td>History-taking</td>
<td>Stop opioids, naloxone</td>
</tr>
<tr>
<td>Acute liver dysfunction</td>
<td>Liver function test, prothrombin time</td>
<td>Conservative treatment, liver transplantation</td>
</tr>
</tbody>
</table>
Neomycin and metronidazole are also poorly absorbed by the intestine, affect urea-producing bacteria, and reduce the generation of ammonia, which improves HE. However, they are not recommended for the management of HE because of their side effects, such as intestinal malabsorption, nephrotoxicity, and ototoxicity for neomycin and peripheral neuropathy for metronidazole. Because ornithine and aspartate are important substrates used to metabolize ammonia to urea and glutamine, the administration of LOLA can lower plasma ammonia concentrations, with produces improvements in HE. For patients with West-Haven criteria grade 1–2 HE, intravenous LOLA can lower the number connection test (NCT)-A time and plasma ammonia concentrations more effectively than placebo. According to a recent RCT, patients treated with the combination of lactulose and intravenous LOLA (30 g/day) had a lower grade of HE within 1–4 days of treatment, with an OR of 2.06–3.04 and a shorter duration until symptom recovery (1.92 vs. 2.50 days, P=0.002), compared with those who received lactulose alone. Oral LOLA can lower the NCT-A time and plasma ammonia concentrations, however, further studies are required to assess its efficacy in managing OHE.

Branched-chain amino acids (BCAAs)

Among cirrhotic patients, the capacity for glycogen storage in the liver decreases along with the reduced liver parenchyma. Therefore, catabolism becomes predominant because protein is required for gluconeogenesis. Because BCAAs, such as valine, leucine, and isoleucine, are absorbed in the peripheral tissue, patients with cirrhosis have a lower concentration of the BCAAs and a higher concentration of the aromatic amino acids in the blood compared with healthy people. Thus, BCAA supplementation inhibits proteolysis and decreases the influx of toxic materials via the blood-brain barrier. Furthermore, it plays an important role in muscle metabolism, leading to glutamine production that is useful for detoxifying ammonia. According to recent meta-analyses, oral BCAAs might be beneficial in managing OHE and should be used as an ancillary pharmacological option. However, intravenous BCAAs have no effect on episodic HE.

Others

Because albumin has great anti-inflammatory and immunomodulatory properties, it might be helpful in improving the overall survival time of patients with decompensated liver cirrhosis. According to recent research in patients with West-Haven criteria grade ≥2 HE, those treated with a combination of lactulose and intravenous albumin (1.5 g/kg/day) showed a better recovery rate within 10 days than those treated with lactulose alone (75% vs. 53.3%, P=0.03) (Table 5).

In addition, polyethylene glycol (PEG), an osmotic laxative, might be tried. Its postulated mechanism of action is flushing ammonia out of the gut, like the nonabsorbable disaccharides. A single RCT comparing PEG (4 liters over 4 hours via oral administration or nasogastric tube) to lactulose only showed it to be superior in terms of clinical improvement over a 24-hour period, documented by a greater decrement in the HE scoring algorithm (Δ 1.5 vs. Δ 0.7, P=0.002) and a shorter median time to resolution (1 day vs. 2 days, P=0.01). However, further studies are re-

| Table 5. Pharmacological options for managing overt hepatic encephalopathy |
|---------------------------------|-----------------------------|
| Non-absorbable disaccharides    | Lactulose (20–30 g) should be administered orally 3–4 times per day (an equivalent daily dose of lactitol is 67–100 g). Goals: it should be administered orally until the patient is having at least 2 bowel movements a day. Thereafter, the dose should be titrated to achieve two to three soft stools per day. If patients cannot take medications orally, administration via nasogastric tube might be tried. Enema with lactulose 200 g and 700 mL water might be performed 3–4 times per day in severe cases. |
| Rifaximin                       | 400 mg three times/day or 550 mg twice/day |
| Oral BCAA                       | 0.25 g/kg/day                |
| Intravenous LOLA                | 30 g/day                    |
| Albumin                         | 1.5 g/kg/day until clinical improvement or for 10 days, maximum |
| Polyethylene glycol             | A substitute for non-absorbable disaccharides |
|                                 | 4 liters orally             |

BCAA, branched-chain amino acid; LOLA, L-ornithine-L-aspartate.
required to assess its efficacy and safety.

Flumazenil, an antagonist of the benzodiazepine receptor, might improve consciousness among patients with severe HE; however, its effect is temporary, and survival time is not improved. Therefore, it is not recommended as a first-line regimen. Nonetheless, it can be used in patients with HE caused by benzodiazepine. Levo-carnitine or sodium benzoate might be effective in managing HE because they can lower plasma ammonia concentrations.

Liver transplantation

Patient with acute liver failure and HE can be considered for liver transplantation because of their poor prognosis. In cases of recurrent OHE, the severity is associated with its overall prognosis, and the overall survival rate after an episode of OHE was 42% and 23% at 1 and 3 years, respectively. Therefore, liver transplantation should be considered for such patients. Furthermore, liver transplantation is also indicated in patients with severe HE who do not respond to the above medical treatments.

**[Recommendations]**

1. Precipitating factors of HE include gastrointestinal bleeding, infection, constipation, infection, excessive intake of protein, dehydration, renal function disorder, electrolyte imbalance, psychoactive medication, and acute hepatic injury. So first, those factors should be recognized and managed. (A1)
2. To manage acute episodic overt HE, non-absorbable disaccharides (e.g., lactulose, lactitol) are recommended. Enema is recommended in severe HE (West Haven criteria grade ≥3) or a clinical situation in which oral intake is inappropriate. (A1)
3. Rifaximin might be combined with non-absorbable disaccharides to treat patients with HE. (B1)
4. Oral BCAA and intravenous LOLA or albumin can be used additionally. (B2)
5. Liver transplantation is indicated in patients with severe HE who do not respond to the medical treatments. (A1)

**Prevention of overt HE**

**Medical therapy**

Among patients with OHE, 50–70% will experience a recurrence within 1-year, so secondary prevention for OHE should be started after the first event. As the first-line therapy, nonabsorbable disaccharides (lactulose, lactitol) should be used. A dose of 30–60 mL of lactulose, allowing 2–3 stools per day, in patients who recovered from acute episodes of OHE significantly reduced the recurrence of OHE (19.6%) compared with the control group (46.8%). In cases of lactulose/lactitol intolerance, rifaximin can be used as single therapy (400 mg tid or 550 mg bid). According to a case-control study that included decompensated liver cirrhosis patients, a median 2 years of rifaximin therapy significantly lowered the recurrence of OHE compared with the control group (31.5% vs. 47%, P=0.034).

A prospective RCT by Bass et al. found that 6-months of rifaximin therapy significantly lowered the recurrence of OHE compared with the placebo group (hazard ratio [HR], 0.42; 95% CI, 0.28–0.64); about 91% of that study population used lactulose concomitantly. Non-absorbable disaccharide and rifaximin combination therapy can reduce the recurrence of OHE more than each single therapy, and it is therefore recommended for recurrent OHE. These medical treatments can effectively prevent OHE recurrence and improve the survival times of patients with OHE.

Long-term treatment with rifaximin raised concerns about the risk of Clostridium difficile (C. difficile) infection, but recent studies found that C. difficile infection was not increased by rifaximin treatment compared with the control group.

Long-term oral BCAA treatment is recommended for patients whose oral diet is insufficient because it can improve symptoms and reduce the recurrence of OHE. In a meta-analysis of 16 RCTs, oral BCAA reduced the recurrence of OHE (HR, 0.73; 95% CI, 0.61–0.88), but the overall survival time did not differ between the two groups.

LOLA can reduce the recurrence of HE. In an RCT including 150 patients, oral LOLA (6 g three times per day) for 6 months significantly reduced the recurrence of OHE (HR, 0.39; 95% CI, 0.17–0.87). Nonetheless, recent meta-analyses have shown that oral LOLA was not more effective than lactulose or rifaximin for OHE prevention.

In patients with intractable ascites, intravenous albumin infusion can prevent OHE. A recent prospective RCT showed that long-term intravenous albumin (40 g per week) infusion significantly lowered the risk of grade 3 or 4 OHE (HR, 0.48; 95% CI, 0.37–0.63) and improved overall survival times (HR, 0.62; 95% CI, 0.40–0.95).

**Education**

A structured educational intervention has been reported to improve patient adherence to prophylactic therapy and reduce readmission with OHE. According to an RCT of 39 patients with a
history of OHE, a 15-minute educational session reduced the risk of OHE-related hospitalization (HR, 0.14; 95% CI, 0.02–0.77). The education of patients and caregivers should include 1) the effects and potential side effects (e.g., diarrhea) of the prescribed medication (lactulose, rifaximin, and so on), 2) the importance of adherence, 3) early symptoms and signs of recurring OHE, and 4) actions to be taken if a recurrence begins.

Nutritional management and exercise

Nutritional deficits and subsequent sarcopenia are known to increase complications, including HE, and lower the overall survival times of cirrhotic patients. Therefore, adequate assessment and intervention for nutritional status are recommended. Because most decompensated cirrhotic patients are malnourished, daily energy intake should be 35–40 kcal/kg, and protein intake should be 1.2–1.5 g/kg. Long-term protein restriction should be avoided because it can induce protein catabolism, hepatic dysfunction, and sarcopenia.

To take in enough energy, small frequent meals (4–6 times per day including a night snack) improve the long-term prognosis for liver cirrhosis patients while preventing sarcopenia, but the direct effect that small meals and a night snack has on OHE prevention has not been fully established.

Exercise can improve the long-term outcomes of cirrhotic patients. In particular, cirrhotic patients usually have decreased skeletal muscle volume because hyperammonemia hinders the synthesis of skeletal muscles. An adequate exercise program can prevent muscle loss, enhance effective ammonia metabolism, and prevent OHE recurrence. However, exercise can temporarily increase the portal pressure in OHE patients, and it could increase the risk of a fall or fracture in malnourished patients. Therefore, adequate nutritional support should precede exercise therapy (Fig. 5).

**Recommendations**

1. A nonabsorbable disaccharide (lactulose, lactitol) or rifaximin, as single or combined therapy, is recommended for the prevention of overt HE recurrence. (A1)
2. Oral branched-chain amino acid or oral LOLA supplementation can prevent the recurrence of overt HE. (B1)
3. Adequate education of patients and caregivers at the time of discharge is needed to reduce the recurrence of overt HE. (B1)
4. Nutritional assessment and management are needed for decompensated liver cirrhosis patients who experienced overt HE. (B1) Long-term protein restriction should be avoided, and adequate energy and protein intakes are necessary. (B1)

**Figure 5.** The treatment and prevention of recurrence of hepatic encephalopathy. PO, per oral; BCAA, branched-chain amino acid; IV LOLA, intravenous L-ornithine-L-aspartate.
Covert HE

Definition
CHE is regarded as the preclinical stage of OHE, and it includes West-Haven criteria grade 1 and MHE, which is the mildest form of HE. 210 It is difficult to diagnose CHE because it can be diagnosed only by psychometric or neurophysiologic examination and is without definite clinical manifestations, such as disorientation or asterixis. Furthermore, it is difficult to clinically distinguish MHE and grade 1 HE. Therefore, MHE and grade 1 HE from the West-Haven criteria are often defined as a single syndrome called CHE. Because the concept of CHE was initiated by ISHEN in 2011, most previous studies have been done on MHE; little research has been done on CHE, including West-Haven criteria grade 1 HE. 210 The prevalence of MHE is 22–78% of patients with liver cirrhosis, although the rate can differ depending on the diagnostic method. 219,302-308 The prevalence of MHE is related to prior episodes of OHE, age, severity of liver disease, and the presence of EVs. 309 In a study using the psychometric HE score (PHES) in a single institution in Korea, MHE was seen in 25.6% of patients with cirrhosis, including 20.2% of those in Child-Pugh A, 42.9% in Child-Pugh B, and 60% in Child-Pugh C. 310

Clinical significance
Patients with CHE have impaired cognitive functions such as attention, executive functions, visuospatial perception, psychomotor speed, and reaction times. 311 Those impaired cognitive functions interfere with daily functioning, such as social interactions, alertness, emotional behavior, sleep, home management, and recreation, and lower the quality of life. 214,304,312 Patients with CHE are at risk of falls and fractures, 313,314 and their poor cognitive performance increases the risk that they will lose their jobs. 315 Therefore, CHE increases the burden on both individual patients and society. CHE is regarded as the preclinical stage of OHE because of the increased risk of progression to OHE, 228,302 and CHE is associated with worsened survival times. 316,317 However, it is difficult to distinguish whether the shortened survival is caused by CHE or hepatic dysfunction.

Diagnosis
To diagnose CHE, the patient must have 1) a disease that can lead to CHE, such as liver cirrhosis or a portosystemic shunt, 2) no other neurological disease, 3) no neurological manifestation such as disorientation or asterixis, and 4) abnormal cognitive or neurophysiologic functioning.

Paper and pencil testing
One of the paper and pencil tests, PHES, consists of five tests (digit symbol test, NCT-A, NCT-B, serial dotting, and line tracing) that measure attention, psychomotor speed, visual perception, and visuo-spatial orientation. 318 The PHES has been widely used to diagnose CHE and has a sensitivity of 96% and a specificity of 100%. 319 It was developed in Germany and has been validated in several countries, including Korea. 310,319-324 It is recommended that at least two of the NCT-A, NCT-B, block design test, and digit symbol test be performed if the full PHES cannot be used due to copyright issues or in places where the PHES has not been validated. 208 The Korean paper and pencil test (KPPT) to evaluate MHE in Korean patients with liver cirrhosis was developed with the support of the Korean Association for the Study of the Liver. 325,326 The KPPT consists of six tests: NCT-A, NCT-B, digit span test (DST), symbol digit modality test (SDMT), word list memory test, and Medical College of Georgia Complex figures. The KPPT short version is configured to be relatively simple to use and contains the NCT-A, NCT-B, DST, and SDMT. A recent prospective multicenter study validated the KPPT short version in Korean patients with liver cirrhosis. 325 The KPPT is available at http://encephalopathy.kr/inspection. 326

Computerized testing
Inhibitory control test (ICT)
The ICT is a computerized test that evaluates attention, response inhibition, and working memory. 306,327 In the ICT, the subject is instructed to respond to alternating patterns of the letters X and Y, called the target. Non-alternating presentations of the letters X and Y, called lures, are randomly planted within the sequence of letters. This test evaluates the response times of the subjects and the response rate to the target and lures. The sensitivity and specificity of the ICT are 87% and 77%, respectively, and it is highly reproducible. 327 However, it has not been validated for Korean patients.

Stroop test
The Stroop test evaluates psychomotor speed and cognitive flexibility using two components (the “off” and “on” states). In the “off” state, subjects match the color of the symbol. In the “on” state, subjects match the color of the word when the color of the word and the meaning of the word are incongruent, which evaluates response inhibition.
The computer-based Stroop test shows a sensitivity of 89.1% and a specificity of 82.1% when using the paper and pencil test as a standard test. A recent prospective multicenter study in the US showed that Stroop test had high sensitivity and acceptable inter-center agreement. In addition, the Stroop test has good test–retest reliability, and it has the advantage that it can be easily administered using a smartphone. A Korean Stroop test was developed, and a recent study showed that the Korean Stroop test is valid for diagnosing MHE (area under the curve, 0.74; 95% CI, 0.66–0.83, \( P < 0.001 \)). The Korean Stroop test is available at http://encephalopathy.or.kr/inspection.

**Neurophysiological testing**

**EEG**

EEG is a test that reflects cerebral cortical neuronal activity. In patients with CHE, a quantitative EEG analysis shows an increase in the relative power of the \( \theta \) band and a decrease in the mean dominant frequency. However, EEG can be affected by various conditions that can affect cortical function. In addition, it requires a technician and a neurologist and is associated with both interobserver and intraobserver variability.

**Critical flicker frequency (CFF)**

CFF measures the frequency at which light begins to flicker noticeably. CFF is highly correlated with paper and pencil testing. In a meta-analysis of nine studies using CFF, the sensitivity and specificity for diagnosing CHE were 61% and 79%, respectively. However, it is not applicable to patients with red-green blindness or Korean patients with cirrhosis because it has not been validated in Korea.

**Other tests**

The animal naming test (ANT) is a semantic fluency test that consists of listing the names of as many animals as possible in 1 minute. In a prospective study conducted in Italy, the sensitivity and specificity of the ANT for diagnosing CHE were 78% and 63%, respectively, when the cut-off was less than 15, and the ANT was a significant predictor for the development of OHE. In a recent prospective study in Germany, the sensitivity and specificity were 31% and 98%, respectively, when the cut-off was less than 15, and they suggested 23 as a cut-off to increase sensitivity.

Nabi et al. reported that a combination of age, sex, and the responses to 4 Sickness Impact Profile (SIP) questions that are highly related to CHE identified patients with CHE with more than 80% sensitivity. However, that test needs to be validated further. Some studies reported that serum cytokines, such as interleukin (IL)-6, IL-17a, interferon-\( \gamma \), and 3-nitrotyrosin, are associated with CHE. However, further studies are needed on the pathophysiology of CHE and the role of the markers in CHE.

**Diagnosis and screening**

CHE has no clinical signs of HE. It can show abnormalities in cognitive functions in various fields, but each field is not reduced to the same extent. In addition, because one test cannot judge the abnormality in all fields and agreement is poor between tests, a combination of at least two tests is recommended for a diagnosis of CHE. For multicenter studies, a paper and pencil test and one computerized or neurophysiologic test are recommended for a CHE diagnosis. A single institution can use one test that has been validated locally.

Because CHE decreases patient quality of life, increases socioeconomic burden, and hastens mortality, it might be necessary to test and diagnose all patients at risk. However, that would increase costs. Therefore, it is advisable to perform a diagnostic test in patients with a history recent of falls or traffic accidents and patients who report a low quality of life or complaints about daily living, such as those who complain of sleep disturbance or a loss of concentration or memory.

Because most patients with CHE are diagnosed at an outpatient clinic, the screening tests should be performable without any special tools and with high sensitivity, such as the four questions from SIP, the ANT, and the Stroop test using a smartphone.

**Treatment**

Most studies about treating CHE were performed in a small number of patients with a short duration of treatment. In addition, most studies have focused on improving cognitive function and quality of life; studies are still needed on extending survival and reducing readmissions or the development of OHE.

As with OHE, it is known that nitrogenous substances, especially ammonia, play a major role in CHE. Therefore, treatments can be given to reduce ammonia. The most studied treatment is lactulose, which showed a marked improvement in cognitive function and quality of life and decreased the development of OHE, compared with the placebo.

Probiotics alter the gut microbiome and inhibit ammonia production in the intestine, thereby improving cognitive function and decreasing the development of OHE. However, studies on the effects of probiotics in patients with CHE have low evidence.
levels. Additional studies are needed to determine the beneficial probiotic species and optimal doses. Rifaximin and nonabsorbable antibiotics also improved cognitive function and quality of life and improved driving ability. However, rifaximin failed to establish non-inferiority over lactulose in non-inferiority studies, and lactulose treatment is more cost-effective than rifaximin therapy. Therefore, further studies on the role of rifaximin in the treatment of CHE are warranted. Although LOLA, BCAA, acetyl L-carnitine, and nutrition therapy have been reported to improve cognitive function, there is still a lack of evidence for those treatments.

[Recommendations]
1. In patients with liver cirrhosis, the KPPT or the Korean Stroop test can be used to diagnose CHE. (B2)
2. Treatment with lactulose (B1) or rifaximin (B2) can be used to improve cognitive function and quality of life in patients with CHE.

HE and health-related quality of life (HRQoL)

HRQoL in patients with cirrhosis is lower than that of patients with chronic liver disease without cirrhosis. The HRQoL of cirrhotic patients with HE is particularly low. Patients with HE suffer from various degrees of altered consciousness, personality changes, impaired intellectual functioning, and neuromuscular dysfunction. Although HE is not immediately life-threatening, it can greatly interfere with a patient’s functioning, social interactions, and sense of well-being. The occurrence of HE is associated with various complications that can also adversely affect HRQoL. Therefore, the independent effect of HE on HRQoL is not easily measured. Because patients with OHE are unaware of their disease (anosognosia), alterations in their behavior and abilities are more easily recognized by the people living with them than by the patients themselves. The presence of OHE negatively affects both mental and physical functioning, whereas MHE mainly has negative effects on mental health. Several studies have shown that the HRQoL of patients with MHE is lower than that of patients without HE. Therefore, we suggest that patients with cirrhosis should be screened for the early detection and treatment of HE to improve their HRQoL.

Measuring of health-related quality of life in patients with HE

HRQoL is measured using self-administered, standardized questionnaires in which patients report their health status. The questionnaires are classified as generic and disease-specific. Because generic questionnaires provide an overview of HRQoL, usually taking into account the physical, mental, and social aspects of a patient’s health status, generic questionnaires have the advantage of depicting the relative impacts of different diseases. However, generic questionnaires have the disadvantage of insensitivity to clinically important changes. Thus, generic questionnaires are often combined with disease-specific questionnaires. The most widely used generic questionnaires for measuring HRQoL are the SIP, Nottingham Health Profile (NHP), and Medical Outcomes Study Short Form-36 (SF-36). The SIP consists of 136 items that measure 12 domains. It requires several minutes to complete, and patients with cognitive dysfunctions sometimes fail to complete it. The NHP measures distress and is useful in patients with moderate or severe disability, but it is not very sensitive to mild disability. The SF-36 is applicable to a wide range of patients, from those with a severe disability to the general population. The SF-36 is easy to complete and has high sensitivity, which makes it the best and most widely used scale in clinical practice. It contains 36 questions that are split into eight domains and provides a physical component summary (PCS) and a mental component summary (MCS) (Supplementary Table 1).

Disease-specific questionnaires have been developed for a variety of chronic diseases, such as renal failure, heart failure, liver cirrhosis, diabetes, and osteoarticular diseases, that greatly affect the HRQoL of patients. Liver-disease-specific questionnaires include the Chronic Liver Disease Questionnaire (CLDQ), Liver Disease Quality of Life (LDQOL), Short Form Liver Disease Quality of Life (SF-LDQOL), and Liver Disease Symptom Index 2.0 (LDISI). The CLDQ comprises 29 questions split into six domains, with domain scores and an overall score presented as 1–7 scales. Higher scores on the CLDQ represent better HRQoL. The CLDQ is short, easily applicable, and correlates with the severity of liver disease. The LDQOL uses the SF-36 and adds 12 liver-specific scales comprising 75 questions. All scales are scored from 0–100, with higher scores representing better HRQoL. The SF-LDQOL uses the SF-36 and adds 36 Likert questionnaires; it is also scored from 1–100. The LDSI uses 18 items to measure the impact and severity of a patient’s liver disease on daily activities in nine areas (Supplementary Table 2).
Influence of HE on health-related quality of life

Although there is a large consensus about the direct and profound effect that HE has on HRQoL, most studies have focused on MHE.208,372,373 A small study about the HRQoL of patients with and without HE compared 18 patients experiencing OHE with 57 patients without a previous episode. Patients with a previous episode of OHE had significantly low SF-36 PCS and MCS scores. However, patients with MHE were affected in only one domain, physical functioning, of the SF-36.374 One study of 160 cirrhotic patients undergoing liver transplantation found that patients with MHE or OHE had a lower MCS than patients without HE.357

Cognitive impairment of patients with HE mainly affects areas that require multiple and complicated functions, such as attention, visuospatial abilities, psychomotor speed, balance, and coordination, rather than language or general intellect. In other words, patients can perform daily activities such as wearing clothes or using the toilet, but their overall planning or cognitive function and exercise performance might suffer.318 Because driving a vehicle requires comprehensive performance and a strategic way of thinking, patients with MHE require attention while driving.375-378 Cirrhotic patients engaged in professions that required sustained attention and motor coordination are more severely affected by MHE than those with jobs that require mainly verbal abilities. In an outpatient cohort with cirrhosis, up to 60% of blue collar workers lost their jobs, versus only 20% of white collar workers.236

A disruption of normal sleep-wake patterns is another early sign of HE.379 and regular sleep is a key indicator of perceived health status. Sleep disturbances are included as relevant items in the assessment of HRQoL in the NHP questionnaire, and sleep disturbances in patients with HE negatively affect HRQoL.380 Patients with MHE report a decrease in the quality of their sleep and in their physical and mental HRQoL.314,381

Psychological status and a patient’s mood can affect the course of a disease and treatment response, and depression affects social functioning, physical abilities, and health status.382 Cirrhotic patients suffer not only from liver disease itself, but also from decreased quality of life in the form of poor work performance and an increased risk of accidents. Therefore, there is a need for extensive social attention and research on public social support systems and economic support for cirrhotic patients.

Effects of treatment on HE and health-related quality of life

Although many studies have aimed at improving HE, relatively few studies have aimed at a significant improvement in the HRQoL of patients with HE.383 Prasad et al.304 first investigated the effect of treatment-related improvements in cognitive function on HRQoL. Patients with MHE treated with lactulose for 3 months showed a significant improvement in their HRQoL on several SIP subscores, particularly in emotional behavior, mobility, sleep/rest, and recreation and pastimes. In an 8-week study of rifaximin therapy in patients with MHE, the patients showed significantly improved scores in both neuro-psychometric performance and the SIP.305 Another study reported that rifaximin therapy to prevent a recurrence in patients with HE favorably affected HRQoL as measured by CLDQ scores.384 Treating OHE patients with oral LOLA385 and MHE patients with acetyl-L-carnitine354 also improved HRQoL. However, a 60-day course of probiotic yogurt supplementation had no significant effects on HRQoL in 25 patients with cirrhosis.346

To date, insufficient studies have been done to establish an association between improved HRQoL and treatments for OHE and MHE. Considering that 10% or more of cirrhotic patients have HE, and 50% of cirrhotic patients with MHE who have not been treated can progress to OHE within 4–24 months,386 it is necessary to change the paradigm of treatment to improve the HRQoL of patients.

[Recommendations]

1. Active diagnosis and treatment of HE improves patient’s health-related quality of life. (A1)
2. Health-related quality of life in patients with HE is assessed by self-administered, standardized questionnaires and can be measured using either generic or disease-specific questionnaires. (B2)

Authors’ contribution

Jae Young Jang (Soonchunhyang University College of Medicine): Introduction Writing and Guidelines General Management
Sang Gyune Kim (Soonchunhyang University College of Medicine): Manuscript writing for introduction of HE
Yeon Seok Seo (Korea University College of Medicine): Manuscript writing for introduction of varices part, surveillance of vari-ces, preventing the formation and progression of EVs
Moon Young Kim (Yonsei University Wonju College of Medicine): Guidelines editing and manuscript organization
Beom Kyung Kim (Yonsei University College of Medicine): Manuscript writing for management of overt HE
Byung Seok Kim (Catholic University of Daegu School of Medicine): Manuscript writing for prevention of first EVs bleeding
Sung Eun Kim (Hallym University College of Medicine): Manuscript writing for HE and health-related quality of life
Ki Tae Suk (Hallym University College of Medicine): Manuscript writing for definition of gastric varices and prevention of primary bleeding, other variceal bleeding and portal hypertensive gastropathy
Do Seon Song (The Catholic University College of Medicine): Manuscript writing for covert HE
Jae Jun Shim (Kyung Hee University College of Medicine): Manuscript writing for management of bleeding from gastric varices and prevention of rebleeding
Seung Kak Shin (Gachon University College of Medicine): Manuscript writing for prevention of EVs rebleeding
Yun Bin Lee (Seoul National University College of Medicine): Manuscript writing for diagnosis and management of acute EVs bleeding
Eun Sun Jang (Seoul National University College of Medicine): Manuscript writing for definition of HE, prevention of overt HE
Dae Won Jun (Hanyang University College of Medicine): Manuscript writing for diagnosis of HE

Acknowledgements
KASL Committee for Revision of the Clinical Practice Guidelines for Liver Cirrhosis: Varices, hepatic encephalopathy, and related complications: Jae Young Jang (Committee Chair, Soonchunhyang University College of Medicine), Sang Gyune Kim (Soonchunhyang University College of Medicine), Yeon Seok Seo (Korea University College of Medicine), Moon Young Kim (Yonsei University Wonju College of Medicine), Beom Kyung Kim (Yonsei University College of Medicine), Byung Seok Kim (Catholic University of Daegu School of Medicine), Sung Eun Kim (Hallym University College of Medicine), Ki Tae Suk (Hallym University College of Medicine), Do Seon Song (The Catholic University College of Medicine), Jae Jun Shim (Kyung Hee University College of Medicine), Seung Kak Shin (Gachon University College of Medicine), Yun Bin Lee (Seoul National University College of Medicine), Eun Sun Jang (Seoul National University College of Medicine), Dae Won Jun (Hanyang University College of Medicine).

Conflicts of Interest
Jae Young Jang: Received grants from Yuhan; received honoraria from BMS, Gilead, Dong-A ST, Yuhan, and Bukwang
Sang Gyune Kim: Received grants from GE healthcare, Samsung Medison, BMS, Samil, Idong; received honoraria from BMS, MSD, Gilead, Dong-A ST, Daewoong; consulted Samsung Medison
Yeon Seok Seo: Received honoraria from Yuhan, Bukwang, Samjin, Gilead, Dong-A ST, Samil, Daewoong, Chongkundang, Abbvie, MSD
Moon Young Kim: Received grants from Samjin, Yuhan, Bukwang; received honoraria from BMS, Gilead, Dong-A ST, Yuhan, Daewoong
Beom Kyung Kim: Received honoraria from Samil, Dong-A ST, Yuhan, Celltrion, Daewoong, BMS, Chongkundang
Byung Seok Kim: Received grants from Gilead, Abbvie, MSD, Dong-A ST, Daewoong, Idong, Chongkundang; received honoraria from Gilead, BMS, Abbvie, Yuhan SK chemical
Sung Eun Kim: Received grants from Daewoong
Ki Tae Suk: Nothing to disclose.
Do Seon Song: Received grants from Alfa Wassermann
Jae Jun Shim: Received grants from Samil; received honoraria from MSD, Yuhan; consulted Gilead
Seung Kak Shin: Nothing to disclose.
Yun Bin Lee: Nothing to disclose.
Eun Sun Jang: Received honoraria from Chongkundang
Dae Won Jun: Received grants from Yuhan, BMS, Gilead, Dong-A ST, Hanwha, Samil, Diagen; received honoraria from Yuhan, BMS, Gilead, Dong-A ST, Hawha, Daewoong

SUPPLEMENTARY MATERIAL
Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

REFERENCES


52. Tripathi D, Ferguson JW, Kochar N, Leithead JA, Therapondos G,

http://www.e-cmh.org
doi:10.3350/cmh.2019.0010n


107. de Franchis R; Baveno V Faculty. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol


133. Kawaoka T, Takahashi S, Aikata H, Azakami T, Saneto H, Takaki

The Korean Association for the Study of the Liver (KASL)
Guideline for varices and hepatic encephalopathy

http://www.e-cmh.org

https://doi.org/10.3350/cmh.2019.0010n


211. Saunders JB, Walters JR, Davies AP, Paton A. A 20-year prospect-

The Korean Association for the Study of the Liver (KASL) Guideline for varices and hepatic encephalopathy


270. Simón-Talero M, García-Martínez R, Torrens M, Augustin S, Gó-


337. Li W, Li N, Wang R, Li Q, Wu H. Interferon gamma, interleukin-6, and -17a levels were correlated with minimal hepatic encephalopathy in HBV patients. Hepatol Int 2015;9:218-223.
351. Sidhu SS, Goyal O, Parker RA, Kishore H, Sood A. Rifaximin vs. lactulose in treatment of minimal hepatic encephalopathy. Liver Int


Introduction of transient elastography in nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide. Although it has become one of the leading causes of cirrhosis and hepatocellular carcinoma in the Western world, the proportion of NAFLD patients developing these complications is rather small. Therefore, current guidelines recommend non-invasive tests for the initial assessment of NAFLD. Among the available non-invasive tests, transient elastography by FibroScan® (Echosens, Paris, France) is commonly used by hepatologists in Europe and Asia, and the machine has been introduced to the United States in 2013 with rapid adoption. Transient elastography measures liver stiffness and the controlled attenuation parameter simultaneously and can serve as a one-stop examination for both liver steatosis and fibrosis. Liver stiffness measurement also correlates with clinical outcomes and can be used to select patients for varices screening. Although obesity is a common reason for measurement failures, the development of the XL probe allows successful measurements in the majority of obese patients. This article reviews the performance and limitations of transient elastography in NAFLD and highlights its clinical applications. We also discuss the reliability criteria for transient elastography examination and factors associated with false-positive liver stiffness measurements. (Clin Mol Hepatol 2020;26:128-141)

Keywords: Liver cirrhosis; Fatty liver; Metabolic syndrome; Obesity; Diagnostic imaging

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide.1 In the United States, nonalcoholic steatohepatitis (NASH), the active form of NAFLD with faster fibrosis progression, has already become one of the leading causes of cirrhosis and hepatocellular carcinoma.2 An updated systematic review and meta-analysis also shows that the prevalence of NAFLD in Asia has increased from 25% between 1999 and 2005 to 34% between 2012 and 2017.3 However, because of the large number of NAFLD patients and that only a minority of patients would eventually develop liver-related morbidity and mortality, non-invasive tests are preferred as initial assessment.
Among the available non-invasive tests, vibration-controlled transient elastography (FibroScan®, Echosens, Paris, France) is commonly used by hepatologists in Europe and Asia. In 2013, the machine was approved by the United States Food and Drug Administration and has rapidly been introduced to different American centers since then. The latest models of transient elastography have different probes (S, M, and XL) to cater for patients with different body build. They also measure both the controlled attenuation parameter (CAP) for hepatic steatosis and liver stiffness for liver fibrosis. In this article, we review the literature on the performance of CAP and liver stiffness measurement (LSM). We discuss the use of M and XL probes, and the reliability criteria of transient elastography examination. Because some but not all studies suggest that hepatic steatosis may confound LSM, we specifically review this issue and highlight other confounding factors.

### LIVER HISTOLOGY

NAFLD is an umbrella term covering the spectrum of disease ranging from nonalcoholic fatty liver (NAFL) to NASH. NAFL is defined as the presence of hepatic steatosis with no hepatocellular injury. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury with or without fibrosis, which is the more progressive form of NAFLD. A subset of patients with NAFLD develop progressive fibrosis, with risk of progression to cirrhosis and hepatocellular carcinoma. Key histological features of NAFLD include steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning and fibrosis. Among the histological features, fibrosis stage has the strongest prognostic significance. The NASH Clinical Research Network system adopts a 5-point fibrosis staging system (0, no fibrosis; 1, perisinusoidal and periportal; 2, bridging fibrosis; 3, cirrhosis).

### CONTROLLED ATTENUATION PARAMETER (CAP)

#### Mechanism

The latest model of transient elastography measures CAP and liver stiffness simultaneously. The former reflects the degree of hepatic steatosis. The typical features of fatty liver on abdominal ultrasonography include bright liver echotexture, deep attenuation of ultrasound signal and vascular blunting. The latter two features are because of the faster attenuation of ultrasound wave amplitude in a steatotic liver. CAP takes advantage of this physical property and estimates the ultrasound attenuation at the central frequency of transient elastography, while assuming a homogeneous fat distribution and an adequate penetration.

#### Performance

Although abdominal ultrasonography is often the first-line investigation for the diagnosis of NAFLD, it is operator-dependent and is insensitive to mild hepatic steatosis. Typically, hepatic steatosis has to involve more than 30% of hepatocytes before ultrasonography can reliably detect fatty liver. Table 1 summarizes studies comparing the performance of CAP against liver histology for the detection of various steatosis grades. Overall, the areas under receiver-operating characteristics curves (AUROC) are 81–84% for ≥S1 (steatosis in at least 5–10% of hepatocytes), 85–88% for ≥S2 (33%), and 86–91% for S3 (66%) steatosis. The reported sensitivities for ≥S1, ≥S2 and S3 are 60–75%, 69-84% and 77–96%, respectively. The corresponding specificities are 76–90%, 75–88% and 72–82%, respectively. A patient-level meta-analysis of nineteen studies involving 2,735 patients shows similar findings, with a pooled sensitivities and specificities of 69% and 82% for ≥S1, 77% and 81% for ≥S2, and 88% and 78% for S3. The optimal cutoffs of CAP are 248 dB/m for S1, 268 dB/m for S2 and 280 dB/m for S3. It should be noted that some studies used patients with other liver diseases as controls without hepatic steatosis. Since such patients underwent liver bi-
Table 1. Performance of controlled attenuation parameter in studies using histology as reference

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>N</th>
<th>Target</th>
<th>Cutoff (dB/m)</th>
<th>Sn (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sasso et al. (2012)</td>
<td>615 CHC patients</td>
<td>S1–S3 (S1 11–33% steatosis)</td>
<td>222</td>
<td>43</td>
<td>93</td>
<td>71</td>
<td>79</td>
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<tr>
<td></td>
<td></td>
<td>S2–S3</td>
<td>233</td>
<td>26</td>
<td>99</td>
<td>77</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S3</td>
<td>290</td>
<td>78</td>
<td>93</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>de Lédinghen et al. (2012)</td>
<td>112 patients</td>
<td>ALD: 6; NAFLD: 28; CHC: 40; miscellaneous: 38</td>
<td>S1–S3 (S1 11–33% steatosis)</td>
<td>263</td>
<td>71</td>
<td>93</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S2–S3</td>
<td>311</td>
<td>57</td>
<td>94</td>
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<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S3</td>
<td>318</td>
<td>87</td>
<td>91</td>
<td>65</td>
<td>97</td>
</tr>
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<td>Myers et al. (2012)</td>
<td>153 patients</td>
<td>Viral hepatitis: 67; NAFLD: 72; other: 14</td>
<td>S1–S3 (S1 5–33% steatosis)</td>
<td>289</td>
<td>68</td>
<td>88</td>
<td>94</td>
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<tr>
<td></td>
<td></td>
<td>S2–S3</td>
<td>288</td>
<td>85</td>
<td>62</td>
<td>56</td>
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<tr>
<td></td>
<td></td>
<td>S3</td>
<td>283</td>
<td>94</td>
<td>47</td>
<td>17</td>
<td>98</td>
</tr>
<tr>
<td>Chan et al. (2014)</td>
<td>238 patients</td>
<td>CHB: 133; NAFLD: 93; other: 12</td>
<td>S1–S3 (S1 5–33% steatosis)</td>
<td>263</td>
<td>92</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Shen et al. (2014)</td>
<td>152 patients</td>
<td>CHB: 100; NAFLD: 52</td>
<td>S1–S3 (S1 5–33% steatosis)</td>
<td>253</td>
<td>89</td>
<td>83</td>
<td>88</td>
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Steatosis was graded as the percentage of hepatocytes with fat: S0 ≤5% or 10%, S1: 5%-33% or 11-33%, S2: 34-66%, S3 ≥67%.
Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; CHC, chronic hepatitis C; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; CHB, chronic hepatitis B; CLD, chronic liver disease.
opsies with other indications, they do not represent a healthy population of subjects. This may have affected the derivation of optimal cutoffs and the evaluation of test performance. Furthermore, the test evaluation is affected by patient composition. Studies based on more obese NAFLD cohorts in Europe and North America usually recommend higher cutoff values for each steatosis grade.30,31

Magnetic resonance imaging (MRI) such as estimated proton density fat fraction (MRI-PDFF) and proton-magnetic resonance spectroscopy is also a leading non-invasive test for steatosis quantification with high sensitivity and specificity.33 MRI is not affected by obesity and has higher success rate of examination than transient elastography.27 Recently, Caussy and colleagues compared CAP and MRI-PDFF in 119 individuals with and without NAFLD.34 The AUROC of CAP to detect MRI-PDFF ≥5% was reasonable at 0.80, whereas that for MRI-PDFF ≥10% was 0.87. The optimal CAP cutoffs for these two MRI-PDFF thresholds were 288 dB/m and 306 dB/m.

While most of the validation studies are cross-sectional in nature, it is important to remember that patients often require serial examinations for disease progression and treatment response. In that regard, MRI-PDFF is precise and can detect small changes in hepatic steatosis over time.35 In contrast, although CAP is reproducible on repeated testing,36 further longitudinal studies should clarify its performance as a monitoring tool.

**Clinical applications**

Although MRI-PDFF has superior performance to CAP, the former is limited by cost and availability.34 In clinical practice, CAP is a reasonable test for the diagnosis of NAFLD, especially as abdominal ultrasonography may be falsely negative when <30% of hepatocytes are steatotic.37 Studies have shown that CAP has strong association with metabolic syndrome, body mass index (BMI), and chronic hepatitis C.19,32,38 CAP is therefore an important and non-invasive method for screening fatty liver in the general population or high-risk population such as patients with type 2 diabetes, obesity and chronic liver diseases (Table 2).39,40

**LIVER STIFFNESS MEASUREMENT (LSM)**

**Mechanism**

During LSM by transient elastography, vibrations of mild amplitude and low frequency (50 Hz) are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues.41 Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness: the stiffer the tissue, the faster the shear wave propagates.42 LSM values range from 1.5 to 75 kPa; lower values indicate a more elastic liver.43 Transient elastography measures liver stiffness in a volume at least 100 times bigger than a biopsy sample, and is therefore far more representative and reliable.43

**Performance**

Transient elastography is painless, rapid and easy to perform at the bedside or in the clinic. It allows rapid and non-invasive estimation of hepatic fibrosis in patients with various chronic liver diseases including chronic hepatitis C,44 chronic hepatitis B,45 and NAFLD.46 Table 3 shows studies on the performance of LSM in NAFLD patients compared with liver biopsy.25,27,29-31,47-51 Overall, the AUROC of LSM for stages F1, F2, F3, and F4 were 0.82, 0.85, 0.94, and 0.96, respectively. For F2–4 fibrosis, the LSM cut-off values range from 6.2 to 11 kPa, with 62–90% sensitivity and 74–100% specificity. For F3–4 fibrosis, the LSM cut-off values range from 8 to 12 kPa, with 84–100% sensitivity and 83–97% specificity. For F4, the LSM cut-off values range from 9.5 to

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<td>- Monitor changes in liver fat (need more data)</td>
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<td><strong>Liver stiffness measurement</strong></td>
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<td>- Estimate severity of liver fibrosis in NAFLD patients</td>
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<td>- Selecting patients for clinical trials or pharmacological treatment</td>
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<td>- Screen for liver fibrosis in the general population of high-risk individuals (e.g., type 2 diabetes and obesity)</td>
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<tr>
<td>- Predict varices needing treatment, hepatocellular carcinoma and liver-related death</td>
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<td>- Monitor changes in liver fibrosis (need more data)</td>
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NAFLD, nonalcoholic fatty liver disease.
Table 3. Performance of liver stiffness measurement in NAFLD studies using histology as reference

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</table>

NAFLD, nonalcoholic fatty liver disease; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.
20 kPa, with 90–100% sensitivity and 75.9–98.4% specificity.

In a meta-analysis of nine studies, the estimates for sensitivity were 87% (95% confidence interval [CI], 84–90%), specificity 91% (95% CI, 89–92%), positive likelihood ratio 11.7 (95% CI, 7.9–17.1), and negative likelihood ratio 0.14 (95% CI, 0.10–0.20). The optimal cutoff varies across studies as it is a tradeoff between sensitivity and specificity and may be influenced by the underlying liver disease.

Magnetic resonance elastography (MRE) is a MRI-based method for quantitatively imaging tissue stiffness. Multiple studies have shown that MRE can be a useful method for the diagnosis of liver fibrosis in patients with NAFLD, even in the early stages. Imajo and colleagues performed a cross-sectional study comparing transient elastography and MRE in 142 Japanese patients with biopsy-proven NAFLD. The AUROC curve in diagnosing liver fibrosis stage 1, 2, 3, and 4 using MRE and transient elastography were 0.80 vs. 0.78, 0.89 vs. 0.82, 0.89 vs. 0.88, and 0.97 vs. 0.92, respectively. The reported sensitivities for F1–4, F2–4, F3–4 and F4 fibrosis were 75% vs. 61.7%, 87.3% vs. 74.5% vs. 85.7% and 90.9% vs. 100%, respectively. The corresponding specificities were 85.7% vs. 100%, 85% vs. 88.7%, 86.9% vs. 83.8% and 94.5% vs. 75.9%, respectively. The findings were confirmed in another study with head-to-head comparison of the two techniques in the United States. The results indicate that MRE has higher diagnostic accuracy in the assessment of liver fibrosis than transient elastography, though the absolute difference is marginal.

LSM by transient elastography is highly reproducible. In the study by Fraquelli and colleagues, 800 transient elastography examinations were performed in 200 patients with chronic liver diseases and the overall interobserver agreement intraclass correlation coefficient (ICC) was 0.98 (95% CI, 0.977 to 0.987). However, increased body mass index (25 kg/m²), steatosis, and fibrosis stage <2 were associated with reduced ICC.

Predicting liver related complications

Transient elastography not only allows early identification of patients with fibrosis and cirrhosis but also plays an important part in predicting complications of compensated advanced chronic liver disease (cACLD), such as gastroesophageal varices, hepatocellular carcinoma and liver-related deaths. Variceal hemorrhage is a common and severe complication of cACLD. Screening for the presence of esophageal varices (EV) with esophagogastroduodenoscopy (EGD; the gold standard) in cirrhotic patients is recommended by current guidelines, but EGD is costly and inconvenient. Many studies have shown that transient elastography has potential value in the prediction of EV. The Baveno VI consensus states that patients with LSM <20–25 kPa and a normal platelet count of ≥150 × 10⁹/L are unlikely to harbor varices needing treatment and may be spared from endoscopy. This notion has since been validated in different settings. In a large multicenter cohort of patients with NASH-related cirrhosis, Petta and colleagues demonstrated the role of probe-specific LSM and platelet count to detect varices needing treatment. The study also suggests the possibility of loosening the criteria (LSM 30 kPa for M probe and 25 kPa for XL probe; platelet count 110 × 10⁹/L) to reduce the number of patients requiring endoscopy further without jeopardizing the false-negative rate (Fig. 1).

In addition to EV, one of the most important complications of liver fibrosis progression is the development of hepatocellular carcinoma. Several studies have proposed that transient elastography can be used to assess the risks of development of hepatocellular

Figure 1. Modified Baveno VI’s criteria to select NAFLD patients for endoscopic screening for varices. NASH, nonalcoholic steatohepatitis; LSM, liver stiffness measurement.
carcinoma, based on the significant correlation between the risk of hepatocellular carcinoma development and the degree of liver fibrosis. A recent systematic review and meta-analysis performed by Singh and colleagues also support these findings. Furthermore, recent studies have shown an association between LSM and survival. Among 2,052 patients with chronic liver diseases, Pang and colleagues reported that LSM by transient elastography had excellent accuracy in predicting the risk of death in patients with chronic liver diseases. On the other hand, CAP does not appear to predict liver-related outcomes. This is in line with liver biopsy studies showing that steatosis is not as important a prognostic marker as the other histological features.

**Clinical applications**

Above all, transient elastography can be performed to estimate severity of liver fibrosis in NAFLD patients at both the primary care and specialist settings. A recent meta-analysis included nine studies consisting of 1,047 NAFLD patients suggests that transient elastography is excellent in diagnosing F3–4 (85% sensitivity, 82% specificity) and F4 fibrosis (92% sensitivity, 92% specificity) and has moderate accuracy for F2–4 fibrosis (79% sensitivity, 75% specificity). Secondly, transient elastography can contribute to select patients for clinical trials or pharmacological treatment. Transient elastography not only has good accuracy and high reproducibility of liver fibrosis, but also has the advantage of being quick, non-invasive, easy to learn and well tolerated by patients, which makes it widely utilize in scientific research. Thirdly, transient elastography can be used to screen for liver fibrosis in the general population of high-risk individuals (e.g., type 2 diabetes and obesity). The overall impact of obesity, type 2 diabetes and other metabolic risk factors on liver fibrosis are greatly underestimated by current practice. In a population-based study among individuals over 45 using transient elastography showed that both BMI >30 kg/m² and type 2 diabetes were significantly associated with liver stiffness ≥8 kPa. In another study of 1,918 patients with type 2 diabetes, 72.8% had fatty liver and 17.7% had high liver stiffness suggestive of advanced fibrosis, highlighting the importance of case finding or even screening in this high risk population. In patients with NASH-related cirrhosis, LSM is a useful tool to predict varices, hepatocellular carcinoma and liver-related death. The Baveno VI criteria and its modifications are good starting points to select patients for endoscopic screening. Last but not least, some studies indicate that transient elastography can be used to monitor fibrosis changes after treatment, though this should be confirmed by further studies using paired liver biopsies.

**M AND XL PROBES**

One of the biggest challenge of transient elastography examination is the lower success rate in obese patients. This is particularly relevant for NAFLD because of its close association with obesity. To cater for this limitation, the manufacturer of transient elastography has produced different probes to cater for patients with different body build. While the M probe is for average adults, the S probe is for children and adolescents and the XL probe is for obese patients. By using a lower frequency (2.5 MHz instead of 3.5 MHz for the M probe), the XL probe measures CAP and liver stiffness at a greater depth (35–75 vs. 25–65 mm). Even in the obese population, the XL probe allows successful measurements in the majority of cases.

Because ultrasound-based transient elastography measures the Young’s modulus and is expected to be affected by ultrasound frequency, prospective studies indeed confirmed that the XL probe would yield a lower liver stiffness value than the M probe when applied on the same patient. Nonetheless, since high body mass index also leads to higher liver stiffness values (see section on confounding factors), the effects of obesity and XL probe on LSM tend to cancel each other. When we applied the M probe in patients with body mass index <30 kg/m² and XL probe in those ≥30 kg/m², the median liver stiffness values by both probes were nearly identical at each fibrosis stage, suggesting that one may adopt the same interpretation when the right probe is used for the right patient (Fig. 2). In our hands, the same CAP cutoffs can also be used for the two probes with similar accuracy. The latest model of transient elastography has an automated probe selection tool that recommends the use of the M or XL probes based on the skin-to-liver capsule distance. When the probe selection tool is followed, again it does not appear that cutoff adjustments are required for the two probes.

**RELIABILITY CRITERIA**

During a transient elastography examination, an operator typically obtains ten measurements. The median values of CAP and liver stiffness reflect the degree of hepatic steatosis and fibrosis, respectively, whereas the interquartile range (IQR) of the ten mea-
surements represents the variability of measurements. Highly variable measurements suggest a difficult examination, suboptimal technique or heterogeneous pathology in the liver parenchyma. According to the original manufacturer recommendations, a reliable LSM is defined as obtaining ten valid measurements, a success rate (number of valid acquisitions divided by the number of attempts) >60%, and an interquartile range-to-median ratio (IQR/M) of ≤0.3. However, subsequent studies have not found success rate to be a determinant of reliable examination.47,79

In a study of 1,165 French patients with chronic liver disease (798 had chronic hepatitis C), Boursier and colleagues proposed new reliability criteria based on both IQR/M and the median liver stiffness values.80 In essence, poorly reliable LSM is defined as IQR/M >0.30 and liver stiffness ≥7.1 kPa for F2–3 fibrosis and ≥12.5 kPa for F4 fibrosis. Because LSM has a high negative predictive value but a modest positive predictive value,81 it is reasonable to consider a patient with a liver stiffness of 7.1 kPa or below as not having significant fibrosis, regardless of the other quality indicators. This approach also has the advantage of reducing the proportion of patients classified as having unreliable examinations.

In a study of 754 patients with chronic liver disease and liver histology (349 had NAFLD), our group showed that an absolute CAP IQR of >40 dB/m with M probe measurement was associated with less reliable diagnosis of hepatic steatosis.82 The finding was confirmed by another study using MRI-PDFF as the reference standard,34 but not in another biopsy-based multicenter United Kingdom study.31 Nevertheless, the latter study only included patients with suspected NAFLD, with only 47 having grade 0 steatosis. That cohort is thus not well suited to determine the reliability criteria for the diagnosis of fatty liver.

CONFOUNDING FACTORS

Well-studied confounding factors for LSM leading to false-positive diagnosis of advanced fibrosis include hepatic congestion,83,84 biliary obstruction,85 amyloidosis,86 and benign and malignant liver lesions.87,88 Probably because of increased portal blood flow, liver stiffness increases after meals by 1–5 kPa.89 The liver stiffness typically peaks at 20 to 40 minutes but may still be increased by 180 minutes.
Acute viral hepatitis and acute exacerbation of chronic viral hepatitis also increase liver stiffness dramatically.\textsuperscript{90,91} In fact, patients with chronic hepatitis B and serum alanine aminotransferase (ALT) elevation to one to five times the upper limit of normal also have higher liver stiffness than those with normal ALT.\textsuperscript{45} However, ALT elevation does not appear to affect LSM in NAFLD patients.\textsuperscript{47} Two reasons may explain this difference. First, NASH is an insidious disease not usually characterized by episodes of acute exacerbations. In general, the degree of hepatic necroinflammation is less prominent in NASH than viral hepatitis or autoimmune hepatitis. Second, the ALT level correlates poorly with histological necroinflammation in NAFLD patients.\textsuperscript{92}

One controversial point is whether severe hepatic steatosis affects liver stiffness. An Italian study showed that severe steatosis increased the false-positive diagnosis of advanced fibrosis by LSM using the M probe in NAFLD patients.\textsuperscript{93} The same applies to patients with high CAP values.\textsuperscript{94} However, it is unclear if the effect is directly due to hepatic steatosis. Other studies have also shown that extreme body mass index is associated with higher liver stiffness.\textsuperscript{95} Recently, our group performed both M and XL probe measurements on 496 patients with biopsy-proven NAFLD and showed that LSM by the XL probe was not affected by severe steatosis.\textsuperscript{78}

Because the above factors represent physical properties of the liver parenchyma, they are expected to affect other kinds of LSMS similarly such as by point-shear wave elastography, 2D-shear wave elastography and magnetic resonance elastography. In contrast, confounding factors for CAP have not been as well studied.

**GUIDELINE RECOMMENDATIONS**

The recommendations of this article are largely in keeping with regional guidelines. The American Association for the Study of Liver Diseases guidelines state that transient elastography and MRE are clinically useful tools for identifying advanced fibrosis in patients with NAFLD, whereas simple fibrosis scores such as the NAFLD fibrosis score and Fibrosis-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis or cirrhosis.\textsuperscript{95} The joint European Association guidelines on NAFLD recommend biomarkers and scores of fibrosis, as well as transient elastography, as acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis or cirrhosis.\textsuperscript{96} They also suggest the use of a combination of biomarkers, scores and transient elastography to monitor fibrosis progression but highlight that this strategy requires validation. Likewise, the joint European and Latin American guidelines on fibrosis assessment endorse screening of liver fibrosis in high risk populations such as patients with metabolic syndrome or type 2 diabetes.\textsuperscript{97} Non-invasive assessment by serum biomarkers or transient elastography can be used as first line procedure for the identification of patients at low risk of severe fibrosis. They also suggest follow-up assessment by serum biomarkers or transient elastography at a 3-year interval. The Asia-Pacific Working Party of NAFLD guidelines also state that non-invasive serum and physical tests have acceptable accuracies when used to measure the fibrotic burden of NAFLD patients.\textsuperscript{98} Notably, none of the guidelines specify cutoffs for CAP and LSM.

As discussed above, the Baveno VI consensus statements recommend the use of transient elastography and platelet count to select patients for endoscopic screening for varices.\textsuperscript{57} They also suggest the use of simple cutoffs of define low and high probability of compensated advanced chronic liver disease.

**CONCLUSIONS**

The development of transient elastography has allowed simultaneous and reasonably accurate assessment of hepatic steatosis and fibrosis. The technique is thus well suited as a point-of-care diagnostic and assessment tool for NAFLD patients. CAP and LSM has been validated across different regions and patient populations with consistent results. LSM not only reflects the degree of liver fibrosis but also predicts portal hypertension, varices requiring treatment, cirrhotic complications and hepatocellular carcinoma. While obesity used to be a common reason for measurement failure, it is possible to obtain valid measurements in the majority of NAFLD patients when the XL probe is used in obese patients. Importantly, with the automatic probe selection tool, operators can apply the same liver stiffness cutoffs when the M and XL probes are used for the right patients. Nonetheless, whether the same applies to CAP interpretation deserves further studies. Well validated reliability criteria for LSM include the requirement of 10 valid measurements and an IQR/M of less than 0.3. Although two studies suggest the IQR also reflects the reliability of CAP, data are conflicting and need further clarification.

As pharmacological treatment for NASH will likely become available in the near future, it is timely to consider the position of non-invasive tests in different settings. Several prospective studies have illustrated the application of simple fibrosis scores, fibro-
sis biomarkers and transient elastography to detect significant liver diseases at primary care setting and selective populations.\textsuperscript{39,98} Notably, it may not be feasible to perform transient elastography for all patients with type 2 diabetes and obesity. A stepwise approach using simple fibrosis scores followed by fibrosis biomarkers or LSM will probably be the way to go but needs to be adjusted for the local setting.

**Author’s contribution**

Xinrong Zhang, Grace Wong and Vincent Wong contributed to the literature review and manuscript preparation.

**Conflicts of Interest**

Grace Wong has served as an advisory committee member for Gilead Sciences and Janssen, and a speaker for Abbott, AbbVie, Bristol-Myers Squibb, Echosens, Gilead Sciences, Janssen and Roche. Vincent Wong has served as an advisory committee member or consultant for 3V-BIO, AbbVie, Allergan, Boehringer Ingelheim, Center for Outcomes Research in Liver Diseases, Echosens, Gilead Sciences, Intercept, Janssen, Novartis, Novo Nordisk, PerfiXion, Pfizer, TARGET-NASH and Terns, and a speaker for Bristol-Myers Squibb, Echosens, Gilead Sciences, and Merck. Both Grace Wong and Vincent Wong have received unrestricted grants from Gilead Sciences.

**REFERENCES**


7. Castera L. Diagnosis of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: non-invasive tests are enough. Liver Int 2018;38 Suppl 1:67-70.


measurement in patients with nonalcoholic fatty liver disease accounting for controlled attenuation parameter values. Hepatology 2017;65:1145-1155.


96. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402.


Drug induced liver injury: East versus West – a systematic review and meta-analysis

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Drug induced liver injury (DILI) may be different in the East compared to the West due to differing disease prevalence, prescribing patterns and pharmacogenetic profiles. To review existing literature on causative agents of DILI in the East compared to the West, a comprehensive literature search was performed on electronic databases: MEDLINE/PubMed, Embase, Cochrane Library and China National Knowledge Infrastructure without language restrictions. Studies which involve patients having DILI and reported the frequency of causative agents were included. A random effects model was applied to synthesize the current evidence using prevalence of class-specific and agent-specific causative drugs with 95% confidence intervals. Of 6,914 articles found, 12 showed the distribution of drugs implicated in DILI in the East with a total of 33,294 patients and 16 in the West with a total of 26,069 DILI cases. In the East, the most common agents by class were anti-tuberculosis drugs (26.6%), herbal and alternative medications (25.3%), and antibiotics (15.7%), while in the West, antibiotics (34.9%), cardiovascular agents (17.3%), and non-steroidal anti-inflammatory drugs (12.5%) were the commonest. For individual agents, the most common agents in the East were isoniazid-rifampicin-pyrazinamide (25.4%), phenytoin (3.5%), and cephalosporin (2.9%) while in the West, amoxicillin-potassium clavulanate combination acid (11.3%), nimesulide (6.3%), and ibuprofen (6.1%) were the commonest. There was significant heterogeneity due to variability in single-centre compared to multi-centre studies. Differences in DILI in the East versus the West both in drug classes and individual agents are important for clinicians to recognize. (Clin Mol Hepatol 2020;26:142-154)

Keywords: Antibiotics, Antitubercular; Chemical and drug induced liver injury; Anti-bacterial agents

INTRODUCTION

Drug induced liver injury (DILI) is defined as liver damage due to drugs, herbal medications or supplements. The pathogenesis of DILI is extremely heterogenous and causes vary from intrinsic hepatotoxicity, mitochondrial toxicity, to immune mediated liver damage related to variations in drug metabolism, genetic differences and HLA susceptibility.¹

DILI has the potential to be fatal with a wide spectrum of liver damage that ranges from mild abnormality to liver fibrosis to acute liver failure. Severe events are relatively rare, but can be catastrophic, particularly once jaundice occurs. This is reflected in Hy's law² in which alanine aminotransferase in serum is increased to over three times the upper limit of normal, and bilirubin is also

Abbreviations:
CI, confidence interval; CVD, cardiovascular disease; DILI, drug induced liver injury; DME, drug metabolizing enzyme; INH₉RIF_PZA, isoniazid-rifampicin-pyrazinamide; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; TB, tuberculosis; WHO-UMC, World Health Organisation-Uppsala Monitoring Center

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Editor: Yoon Jun Kim, Seoul National University College of Medicine, Korea

Received: Jul. 23, 2019 / Revised: Aug. 29, 2019 / Accepted: Sep. 5, 2019

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increased to over twice the upper limit of normal. Those who fulfill this “rule” have over 10% risk of mortality or liver transplantation.

The mainstay of treatment is withdrawal of the offending agent. In mild to moderate cases, this is followed by eventual resolution of the liver damage, but in severe cases, liver transplantation may be necessary.

There are very few studies on the incidence of DILI worldwide. The main difficulty is to ascertain the denominator, i.e., the number of individuals receiving a culprit drug. True prevalence studies are prospective community based studies with well characterised reporting systems, low dropout and loss to follow up. Consequently, there are very few population based studies.

So far the ‘best’ available population based survey was performed in Nievre, France, 2002 where the local population had only a few hospitals for their healthcare, and almost all the doctors participated in the study using a standard protocol following the International Consensus Meeting Proposal. The annual incidence rate of DILI was 13.9±2.4 per 100,000 inhabitants. In a population based study in Iceland recently, the crude annual incidence rate of DILI was 19.1 per 100,000 inhabitants, with a crude hospitalization rate of 4.4 per 100,000. A Spanish study in 2005 estimated the annual incidence of hepatotoxicity to be 3.4 per 100,000 inhabitants with crude hospitalization rate of 1.6 per 100,000 inhabitants. In the East, a prospective study from Korea in 2012 involving 17 referring university hospitals with defined criteria based on World Health Organisation-Uppsala Monitoring Center (WHO-UMC) extrapolated crude annual incidence rate to be 12 per 100,000. Based on the above limited data available, it appears that there is not a major difference in incidence of DILI between the East and the West. However it is difficult to make firm conclusions based on the scanty data available, and there is a definite need for well conducted prospective population studies, particularly from the East.

Is drug induced liver injury different in the East compared to the West? One may speculate that Asians have a different pharmacogenetic and immunological profile, and may therefore handle drugs differently. An additional problem in Asia is the widespread use of herbal and alternative medicines, the safety of which is poorly defined.

Consequently, there is an unfilled knowledge gap with regards to differences in Eastern compared to Western DILI with regards to causative agents. We aimed to determine whether agents causing DILI in the East was different from the West by performing meta-analyses of studies of DILI reported in the East, and those in the West, with regards to the most frequently reported agents. Since the reporting frequencies of DILI are most affected by reporting bias, we took measures to reduce bias.

METHODS

Literature search and eligibility criteria

A comprehensive literature search was performed on electronic databases: MEDLINE/PubMed, Embase, Cochrane Library and China National Knowledge Infrastructure until 31 March 2016. The specific concepts used in the search strategy were “drug induced liver injury”, “incidence” and “prevalence”. We searched these terms in combination with the conducted literature search by Medical Subject Headings or Emtree terms, and free text terms. There were no restrictions on language. All the bibliography listed in review papers and included publications were also checked. Two investigators (E.X.S.L. and S.G.L.) independently screened for eligible studies based on pre-defined eligibility criteria. Inclusion criteria were cross sectional or cohort studies which involve patients having DILI and which reported the frequency of the causative agents. For studies that had published duplicate results with accumulating numbers of patients, only the most recent or complete reports were included. Exclusion criteria were studies which sampled less than 200 subjects, did not provide sufficient information, technical reports, editorials, letters to the editor, and case reports. Any discrepancies regarding whether articles met selection criteria were resolved by consensus.

Data extraction and risk of bias assessment

The following data were extracted (E.X.S.L. and S.G.L.) from the included studies: 1) study characteristics (publication year, country of population, causality assessment criteria and study design); and 2) frequency of class-specific and agent-specific causative drugs. To reduce bias, frequency of agent specific causative drugs were only included if they were present in at least three studies.

The quality of each study was evaluated by two independent investigators (Z.Q. and E.C.). Quality of sampling (i.e., source of sampling and sampling methods) and quality of measurement (i.e., causality assessment criteria and consistency of criteria application) were assessed to determine the study quality. Any disagreement in quality assessment was resolved by discussion and consensus.
Statistical analysis

A random effects model was applied to synthesize the current evidence using prevalence of class-specific and agent-specific causative drugs with 95% confidence interval (CI). To assess the potential heterogeneity, we calculated the $I^2$ for each of analysis. We also planned a sub-group analysis on single-center and multi-center study to explore the potential source of heterogeneity, as it was possible that single-center studies may lead to higher risk of bias compared to multi-center studies. Potential small-study effects and publication bias was assessed by a funnel plot. The estimated prevalence was plotted against the inverse of the standard error of the prevalence as a measure of precision reflecting the effect size. The Egger’s test was conducted with the null hypothesis that symmetry exists in the funnel plot. Statistical analyses were performed using Comprehensive Meta Analysis 3.3 (Biostat, Englewood, NJ, USA) and StataMP 15.1 (StataCorp LLC, College Station, TX, USA).

RESULTS

A total of 6,914 studies were found using the search strategy but after exclusions only a total of 28 studies (Fig. 1, Table 1) were included for analysis. The causative agents were listed by class or as individual agents. The results were reported as event rates and 95% CIs, then were ranked based on the frequency of events, either as a drug class or as individual class. The frequencies and event rates were shown between studies reported from Western countries and Eastern countries which were present in at least three studies. Since the studies were independently conducted no direct differences could be compared.

Frequency of DILI by class and individual agents: West versus East

In Figure 2, summary pooled estimates of the frequency of causative agents reported in Eastern and Western studies were shown by drug class in a descending order of number of causative events present in at least three studies. Forrest plot of classes of agents can be viewed in the supplementary material (Supplementary Fig. 1, 2). Drug classes
<table>
<thead>
<tr>
<th>No</th>
<th>Study</th>
<th>Country/region</th>
<th>Causality assessment for DILI</th>
<th>Time periods</th>
<th>Publication type (report, journal article, abstract)</th>
<th>Hospital-/registry-based</th>
<th>Drug classification type (single agent or class)</th>
<th>Total number of DILI records</th>
<th>Included acetaminophen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Andrade et al. (2005)</td>
<td>Spain</td>
<td>CIOMS/RUCAM Clinical judgement, OMS by 3 independent experts</td>
<td>Apr 1994 to Aug 2004</td>
<td>Prospective</td>
<td>Regional registry</td>
<td>Both</td>
<td>461</td>
<td>Yes 13 cases</td>
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<tr>
<td>2</td>
<td>Bessone et al. (2016)</td>
<td>Latin America</td>
<td>CIOMS/RUCAM</td>
<td>2011 to 2014</td>
<td>Prospective</td>
<td>Latin American registry</td>
<td>Single</td>
<td>206</td>
<td>Yes but 0 cases</td>
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<tr>
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<td>Björnsson et al. (2013)</td>
<td>Iceland</td>
<td>CIOMS/RUCAM</td>
<td>Mar 2010 to Feb 2012</td>
<td>Prospective</td>
<td>National</td>
<td>Both</td>
<td>96</td>
<td>No (specifically excluded)</td>
</tr>
<tr>
<td>4</td>
<td>Chalasani et al. (2015)</td>
<td>USA</td>
<td>DILIN methods</td>
<td>Sep 2004 to May 2013</td>
<td>Observational longitudinal</td>
<td>National</td>
<td>Both</td>
<td>899</td>
<td>No (specifically excluded)</td>
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<td>6</td>
<td>De Valle et al. (2006)</td>
<td>Swedish outpatient clinic</td>
<td>CIOMS</td>
<td>1995 to 2005</td>
<td>Retrospective review of case records</td>
<td>Both</td>
<td>77</td>
<td>Not mentioned</td>
<td></td>
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<td>de Abajo et al. (2004)</td>
<td>UK</td>
<td>Description like RUCAM but not specifically stated</td>
<td>1994 to 1999</td>
<td>Population based case control (5,000 controls)</td>
<td>GP research database in the UK</td>
<td>Both</td>
<td>128</td>
<td>Yes: 12 from acetaminophen</td>
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<td>Carey et al. (2008)</td>
<td>US Mayo (inpatients)</td>
<td>CIOMS</td>
<td>1998 to 2006</td>
<td>Retrospective search with codes</td>
<td>Inpatient visits at Mayo Hospital</td>
<td>Both</td>
<td>40</td>
<td>Yes: 40 had DILI, 27 from acetaminophen</td>
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<td>12</td>
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<td>Switzerland</td>
<td>CIOMS</td>
<td>Jan 1996 to Dec 2000</td>
<td>Medical records review</td>
<td>Pharmacoepidemiological databases</td>
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<td>Sabaté et al. (2007)</td>
<td>Barcelona Spain 12 hospitals</td>
<td>Jaundice, ALT AST</td>
<td>Jan 1993 to Dec 1999</td>
<td>Multi centre prospective case control</td>
<td>12 hospitals 2,700,000</td>
<td>Drugs</td>
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<td>Country/region</td>
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<td>Time periods</td>
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<td>Hospital-/registry-based</td>
<td>Drug classification type (single agent or class)</td>
<td>Total number of DILI records</td>
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<tr>
<td>14</td>
<td>Sistanizad Peterson(^3) (2013)</td>
<td>Tasmania, Australia</td>
<td>CIOMS</td>
<td>Jun 2008 to July 2009</td>
<td>Retrospective</td>
<td>1 major hospital serving 250,000 people</td>
<td>Drugs</td>
<td>17</td>
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<td>Description like RUCAM but not specifically stated</td>
<td>1993 to 2002</td>
<td>Retrospective review of records</td>
<td>1 tertiary care hospital</td>
<td>Both</td>
<td>32</td>
<td>Yes but 0 cases</td>
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<tr>
<td>16</td>
<td>Vega et al.(^25) (2017)</td>
<td>Delaware, US</td>
<td>CIOMS</td>
<td>2014</td>
<td>Prospective</td>
<td>DILN</td>
<td>Both</td>
<td>23</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>Suk et al.(^4) (2012)</td>
<td>Korea</td>
<td>RUCAM</td>
<td>May 2005 to May 2007</td>
<td>Prospective</td>
<td>17 referral hospitals (nationwide)</td>
<td>Class</td>
<td>371</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>Kwon et al.(^26) (2012)</td>
<td>Korea</td>
<td>WHO-UMC</td>
<td>Jan 2007 to Dec 2008</td>
<td>Retrospective registry of spontaneous reports of adverse drug reactions</td>
<td>9 regional pharmacovigilence centres in Korea (nationwide)</td>
<td>Both</td>
<td>567</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>Ou et al.(^28) (2015)</td>
<td>China</td>
<td>CIOMS</td>
<td>Jan 2011 to Dec 2014</td>
<td>Retrospective review of inpatient records</td>
<td>Inpatients - 1 hospital</td>
<td>Both</td>
<td>361</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>Lee et al.(^29) (2012)</td>
<td>Taiwan</td>
<td>ICD–9 code (case cross over comparison of diagnosis)</td>
<td>1997 to 2004</td>
<td>Retrospective</td>
<td>Population based database (insurance)</td>
<td>Both</td>
<td>4,857</td>
<td>Unclear</td>
</tr>
<tr>
<td>22</td>
<td>Takikawa et al.(^30) (2009)</td>
<td>Japan</td>
<td>DDW-J 2004</td>
<td>Jan 1997 to Dec 2006</td>
<td>Retrospective</td>
<td>29 facilities (nationwide)</td>
<td>Class</td>
<td>1,676</td>
<td>Yes</td>
</tr>
<tr>
<td>23</td>
<td>Huang et al.(^31) (2013) (abstract)</td>
<td>Taiwan</td>
<td>RUCAM</td>
<td>Unclear</td>
<td>Retrospective</td>
<td>6 medical centres across Taiwan</td>
<td>Class</td>
<td>1,099</td>
<td>Yes</td>
</tr>
<tr>
<td>24</td>
<td>Sobhonsldisuk et al.(^32) (2016)</td>
<td>Thailand</td>
<td>ICD-10 (toxic liver disease)</td>
<td>2009 to 2013</td>
<td>Retrospective</td>
<td>Population based database (nationwide) (DILN Taiwan)</td>
<td>Single</td>
<td>589</td>
<td>Yes</td>
</tr>
</tbody>
</table>
were listed as antibiotics, anti-epileptics, anti-tuberculosis (TB) drugs, cardiovascular (CVD) agents, gastrointestinal agents, herbal and supplements, lipid lowering agents, non-steroidal anti-inflammatory drugs (NSAIDs), and psychotropic drugs. The event rate and 95% CIs for each study and the pooled analysis for each drug class was shown. Significant heterogeneity was shown for pooled analysis of some drug classes and in other drug classes, when the number of studies was less than four, it was not possible to generate statistical heterogeneity (Fig. 2).

In Figure 3A, summary pooled estimates of the frequency of individual agents reported in Eastern studies and in Figure 3B, summary pooled estimates of individual agents reported in Eastern studies were shown. Forrest plots of the individual agents can be viewed in the supplementary material (Supplementary Fig. 3). The event rate and the 95% CIs are shown for each study and the pooled estimate. Only four drugs as individual agents were found from a search of Eastern studies, as most of the studies reported only drug classes in contrast to Western studies where there were many more reports of individual drugs. Significant heterogeneity was seen for acetaminophen, amoxicillin-clavulanate acid, diclofenac, flucloxacillin, heparin, and minocycline for Western studies, while in Eastern studies, heterogeneity was seen in acetylaminoeph, amoxicillin-clavulanate acid, diclofenac-flucloxacillin, heparin, and minocycline.

In Table 2, a summary table of the ranking of number of causative events of the class of agents implicated in DILI from Western studies and Eastern studies are shown. In Western studies antibiotic DILI ranked the highest with 1,161 events and a prevalence of 34.9% (95% CI, 25.4-45.1%) while anti-TB drugs was ranked the highest for Eastern studies with 563 events and a prevalence of 26.6% (95% CI, 13.1-42.9%). For the second ranked DILI in the West, CVD agents had 392 events and a prevalence of 17.3% (95% CI, 7.8-29.5%), while in the East, herbs and supplements ranked second with 914 events and a prevalence of 25.3% (95% CI, 12.5-40.6%). In the West, psychotropic drugs ranked third with 25.3% (95% CI, 12.5-40.6%) and in the East, antibiotics ranked third with 25.4% (95% CI, 12.5-40.6%). The event rate and the 95% CIs are shown for each study and the pooled estimate. Only four drugs as individual agents were found from a search of Eastern studies, as most of the studies reported only drug classes in contrast to Western studies. Significant heterogeneity was seen for acetaminophen, amoxicillin-clavulanate acid, diclofenac-flucloxacillin, heparin, and minocycline for Western studies, while in Eastern studies, heterogeneity was seen in acetylaminoeph, amoxicillin-clavulanate acid, diclofenac-flucloxacillin, heparin, and minocycline.

In Table 3, a summary table of the ranking of the number of DILI records included in Eastern studies and Western studies are shown. While anti-TB agents are also antibiotics, they were classed separately since they were often quite specific and used in combination. While anti-TB agents are also antibiotics, they were classed separately since they were often quite specific and used in combination. In Table 3, the summary table of the ranking of the number of DILI records included in Eastern studies and Western studies are shown. While anti-TB agents are also antibiotics, they were classed separately since they were often quite specific and used in combination.
Figure 2. (A) DILI by drug class of Eastern studies. Summary pooled estimates are shown as ES and 95% CIs. Where I² and P-values are not shown, this indicates <4 studies and statistical heterogeneity could not be assessed. (B) DILI by drug class of Western studies. Summary pooled estimates are shown as ES and 95% CIs. Where I² and P-values are not shown, this indicates <4 studies and statistical heterogeneity could not be assessed. ES, estimate of proportion; CI, confidence interval; DILI, drug induced liver injury.

<table>
<thead>
<tr>
<th>Study</th>
<th>ES (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TB (n=26,452)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=98.5%, P=0.000)</td>
<td>0.275 (0.185, 0.376)</td>
</tr>
<tr>
<td>Anti-fungal (n=26,211)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.015 (0.013, 0.016)</td>
</tr>
<tr>
<td>Antibiotic (n=28,168)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=97.5%, P=0.000)</td>
<td>0.161 (0.112, 0.217)</td>
</tr>
<tr>
<td>Antineoplastics (n=25,040)</td>
<td>Subtotal (I² not assessable)</td>
</tr>
<tr>
<td>Antithyroid (n=24,473)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.027 (0.025, 0.029)</td>
</tr>
<tr>
<td>Antivirals (n=24,729)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.024 (0.014, 0.057)</td>
</tr>
<tr>
<td>CVD agents (n=26,026)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.052 (0.014, 0.112)</td>
</tr>
<tr>
<td>Gastro agents (n=26,026)</td>
<td>Subtotal (I² not assessable)</td>
</tr>
<tr>
<td>Herbal and supplements (n=28,265)</td>
<td>Subtotal (I²=98.9%, P=0.000)</td>
</tr>
<tr>
<td>Lipid-lowering (n=25,637)</td>
<td>Subtotal (I² not assessable)</td>
</tr>
<tr>
<td>NSAIDs (n=27,767)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=97.8%, P=0.000)</td>
<td>0.059 (0.042, 0.079)</td>
</tr>
<tr>
<td>Psychotropic (n=26,766)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=98.2%, P=0.000)</td>
<td>0.073 (0.030, 0.132)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>ES (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TB (n=694)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.079 (0.060, 0.101)</td>
</tr>
<tr>
<td>Antibiotic (n=2,429)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=92.5%, P=0.000)</td>
<td>0.322 (0.246, 0.401)</td>
</tr>
<tr>
<td>Antineoplastics (n=1,544)</td>
<td>Subtotal (I²=74.3%, P=0.000)</td>
</tr>
<tr>
<td>CVD agents (n=1,684)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=96.6%, P=0.000)</td>
<td>0.168 (0.075, 0.289)</td>
</tr>
<tr>
<td>Gastro agents (n=1,025)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.030 (0.020, 0.041)</td>
</tr>
<tr>
<td>Herbal and supplements (n=1,467)</td>
<td>Subtotal (I² not assessable)</td>
</tr>
<tr>
<td>Lipid-lowering (n=591)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.050 (0.015, 0.100)</td>
</tr>
<tr>
<td>NSAIDs (n=1,932)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=97.0%, P=0.000)</td>
<td>0.137 (0.051, 0.252)</td>
</tr>
<tr>
<td>Psychotropic (n=824)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I²=75.4%, P=0.003)</td>
<td>0.126 (0.077, 0.185)</td>
</tr>
<tr>
<td>Antiepileptics (n=254)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (I² not assessable)</td>
<td>0.031 (0.012, 0.057)</td>
</tr>
</tbody>
</table>
Figure 3. (A) DILI by individual agents in Eastern studies. Summary pooled estimates are shown as ES and 95% CIs. Where $I^2$ and $P$-values are not shown, this indicates <4 studies and statistical heterogeneity could not be assessed. (B) DILI by individual agents in Western studies. Summary pooled estimates are shown as ES and 95% CIs. Where $I^2$ and $P$-values are not shown, this indicates <4 studies and statistical heterogeneity could not be assessed.

ES, estimate of proportion; CI, confidence interval; INH_RIF_PZA, isoniazid-rifampicin-pyrazinamide; DILI, drug induced liver injury.
causative events of the individual agents implicated in DILI from Western studies and Eastern studies are shown. There was a much larger number of individual agents described in Western studies (n=9) compared to Eastern studies (n=4). This is mainly due to Eastern studies describing classes of agents rather than individual agents. The top ranked individual agent in the West was amoxicillin-clavulanate with 356 events and a prevalence of 11.3% (95% CI, 8.4–14.8%) while in the East was INH_RIF_PZA with 25.4% (95% CI, 8.3–47.7%). Nimesulide with 77 events and prevalence of 6.3% (95% CI, 0.87–15.9%) was ranked second in the West and phenytoin with 25 events and a prevalence of 3.5% (95% CI, 0.6–8.2%) in the East.

Potential heterogeneity and publication bias

Almost all the forest plots showed significant statistical heterogeneity. One source of heterogeneity was whether the data source was a multi-centre or single centre study. It is possible that single centre studies may lead to higher risk of bias compared to multi-centre studies. There were significant differences in prevalence for individual drugs. This was especially true for Western studies in particular, such as the case of acetaminophen where multi-centre studies reported an event rate of 7% (95% CI, 0.9–37.1%) compared to 67.5% (95% CI, 51.7–80.1%) for single centre studies (Supplementary Table 1). For Eastern studies, only amoxicillin-clavulanate and INH_RIF_PZA were represented as both multi-centre and single centre studies, and differences were relatively minor. When we examine the differences in class of drugs comparing multi-centre versus single centre studies in Western studies, there were notable differences in prevalence of antibiotics 27.1% (95% CI, 19.0–36.9%) for multi-centre studies, and 42.1% (95% CI, 27.6–58.2%) for single centre studies and NSAIDs 9.1% (95% CI, 2.8–25.5%) for multi-centre studies, and 17.7% (95% CI, 7.9–35.1%) for single centre studies. In Eastern studies, notable differences were seen in prevalence of antibiotic related DILI, 11.7% (95% CI, 6.9–19.3%) for multi-centre studies, and 26.1% (95% CI, 6.5–64.1%) for single centre studies, and lipid-lowering agents 2.4% (95% CI, 0.7–8.1%) for multi-centre studies, and 11.4% (95% CI, 0.4–78.9%) for single centre studies (Supplementary Table 1). Given that funnel plots generally require at least 10 studies for Egger’s test, we selected Antibiotics for the plot generation as it was the most reported medication class among the included studies. From the Egger’s tests, we found that both P-values were larger than 0.05, indicating that current

Table 2. Summary of ranks by class and single agents by class (with all studies)

<table>
<thead>
<tr>
<th>Rank</th>
<th>West</th>
<th>No. of studies</th>
<th>DILI event</th>
<th>Total DILIs</th>
<th>Prevalence (%), range</th>
<th>East</th>
<th>No. of studies</th>
<th>DILI event</th>
<th>Total DILIs</th>
<th>Prevalence (%), range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antibiotics</td>
<td>15</td>
<td>1,167</td>
<td>3,613</td>
<td>34.9 (25.4, 45.1)</td>
<td>Anti-TB</td>
<td>4</td>
<td>563</td>
<td>2,340</td>
<td>26.6 (13.1, 42.9)</td>
</tr>
<tr>
<td>2</td>
<td>CVD agents</td>
<td>6</td>
<td>392</td>
<td>2,868</td>
<td>17.3 (7.8, 29.5)</td>
<td>Herbal and sup</td>
<td>8</td>
<td>914</td>
<td>4,164</td>
<td>25.3 (12.5, 40.6)</td>
</tr>
<tr>
<td>3</td>
<td>Psychotropic</td>
<td>7</td>
<td>161</td>
<td>1,512</td>
<td>13.1 (6.8, 21.0)</td>
<td>Antibiotics</td>
<td>9</td>
<td>554</td>
<td>4,380</td>
<td>15.7 (9.0, 23.9)</td>
</tr>
<tr>
<td>4</td>
<td>NSAIDs</td>
<td>10</td>
<td>307</td>
<td>3,252</td>
<td>12.5 (6.8, 19.8)</td>
<td>Psychotropic</td>
<td>5</td>
<td>251</td>
<td>2,665</td>
<td>8.2 (4.4, 12.8)</td>
</tr>
<tr>
<td>5</td>
<td>Herbal and sup</td>
<td>4</td>
<td>184</td>
<td>2,094</td>
<td>6.7 (1.2, 16.0)</td>
<td>NSAIDs</td>
<td>5</td>
<td>256</td>
<td>3,666</td>
<td>4.8 (2.2, 8.2)</td>
</tr>
</tbody>
</table>

Table 3. Summary of ranks by class and single agents by single agents

<table>
<thead>
<tr>
<th>Rank</th>
<th>West</th>
<th>No. of studies</th>
<th>DILI event</th>
<th>Total DILIs</th>
<th>Prevalence (%), range</th>
<th>East</th>
<th>No. of studies</th>
<th>DILI event</th>
<th>Total DILIs</th>
<th>Prevalence (%), range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amoxicillin-clavulanate</td>
<td>15</td>
<td>356</td>
<td>2,977</td>
<td>11.3 (8.4, 14.8)</td>
<td>INH_RIF_PZA</td>
<td>4</td>
<td>348</td>
<td>1,270</td>
<td>25.4 (8.3, 47.7)</td>
</tr>
<tr>
<td>2</td>
<td>Nimesulide</td>
<td>4</td>
<td>77</td>
<td>1,485</td>
<td>6.3 (0.87, 15.9)</td>
<td>Phenytoin</td>
<td>4</td>
<td>25</td>
<td>1,270</td>
<td>3.5 (0.6, 8.2)</td>
</tr>
<tr>
<td>3</td>
<td>Ibuprofen</td>
<td>5</td>
<td>68</td>
<td>1,389</td>
<td>6.1 (2.8, 10.4)</td>
<td>Cephalosporin</td>
<td>3</td>
<td>17</td>
<td>1,241</td>
<td>2.9 (0.0, 10.1)</td>
</tr>
<tr>
<td>4</td>
<td>INH_RIF_PZA</td>
<td>8</td>
<td>109</td>
<td>2,027</td>
<td>4.6 (3.2, 6.2)</td>
<td>Carbamazepine</td>
<td>3</td>
<td>61</td>
<td>1,241</td>
<td>1.3 (0.4, 2.7)</td>
</tr>
<tr>
<td>5</td>
<td>Diclofenac</td>
<td>8</td>
<td>75</td>
<td>2,588</td>
<td>3.7 (1.8, 6.2)</td>
<td>Valproate</td>
<td>3</td>
<td>37</td>
<td>909</td>
<td>0.3 (0.0, 1.8)</td>
</tr>
</tbody>
</table>

DILI, drug induced liver injury; TB, tuberculosis; CVD, cardiovascular; NSAIDs, non-steroidal anti-inflammatory drugs.
DISCUSSION

Studies of DILI have reported varying frequency of implicated agents. Almost all of these studies come from the West where there are well established networks and infrastructure for DILI. From our systematic review, we can conclude that the agents commonly implicated in the East are quite different to those from the West. Ranking of number of causative events show that in the West, amoxicillin-clavulanate, nimesulide and ibuprofen are the common agents implicated in DILI while in the East, INH_RIF_PZA, phenytoin and cephalosporins are the commonly reported. INH_RIF_PZA is common in Eastern DILI, but is less frequent in the West.

Examining the event frequencies of classes of DILI agents are also revealing. Antibiotics 34.9%, are the most frequent class of agent in the West but only the third most frequent 15.7% in the East, and is reflected in the lower frequency by almost half. An increasingly important DILI group is herbs and supplements, for which Eastern studies show a frequency of 25.3% compared to Western studies showing a significantly lower frequency of 6.7%.

The herbs implicated are also likely to be different in the West compared to the East. In a review of herbal hepatotoxicity, Teschke and Eickhoff compiled a comprehensive overview of herbal preparations implicated. By extracting the source reference, we compiled the top 10 common herbs implicated in the East versus the West (Table 4). The most common herb causing hepatotoxicity in the East was Tu San Qi (Gynura segetum, n=164 cases) while the most common herbal hepatotoxicity in the West was Lu Cha (Camellia Sinensis, n=133 cases) or Chinese Green Tea (Table 4).

Unfortunately, in the case of most herbal DILI, it may be impossible to identify the particular chemical that is causing the liver damage. Moreover, many herbal preparations may contain undeclared adulterants, which could also contribute to liver damage.

By using the frequency of reported DILI comparing Western reports to that of Eastern reports we are able to obtain an overview of the common drugs implicated and a pooled estimate of their frequency. Nonetheless, the study’s main limitation is that of reporting bias. Potential sources of bias were reduced by examining subgroups using multicentre rather than single centre studies. These were sources of heterogeneity with particular drugs such as acetaminophen. In the case of acetaminophen, this may be due to a classification problem since it may be considered a drug causing liver toxicity and tends to be treated separately from DILI as this has been excluded from the prospective DILIN study in the USA.

The certainty of pooled estimates are reflected by the narrowness of the CIs. Although this can be a little subjective, we can be
more certain when CIs are relatively narrow. In this regard, Western reports of antibiotics show an event frequency of 34.9% (95% CI, 25.4–45.1%) but when examining single agents of antibiotics, amoxicillin-clavulanate 13.1% (95% CI, 10.6–16.2%), and nitrofurantoin 5.1% (95% CI, 3.3–7.8%) provide more reliable estimates while flucloxacillin 12.2% (95% CI, 4.9–27.5%), and minocycline 5.9% (95% CI, 1.4–21.0%) have wider CIs. In other drug classes of Western DILI, anti-TB drugs 8.2% (95% CI, 6.3–10.5%) and anti-neoplastic drugs 4.7% (95% CI, 2.8–7.7%) have reasonably narrow CIs. With regards to Eastern DILI by drug class, NSAIDs 4.8% (95% CI, 2.2–8.2%) has reasonable CIs and for the single agents, carbamazepine 1.8% (95% CI, 0.7–4.7%) has reasonable CIs. Nonetheless, despite the wider CIs in some instances, the overall findings and rankings of both classes of agents and individual agents provides a picture of differences in DILI between East and West.

Could the differences between Western and Eastern DILI be explained by pharmacogenetic differences? One well studied field is TB drug related DILI. A meta analysis by Cai et al.10 studied the association between drug metabolizing enzyme (DME) gene polymorphisms and the risk of TB drug related DILI. Four DME genes were studied (NAT2, CYP2D1, GSTM1, and GSTT1) through 38 studies involving more than 2,000 patients. TB drug related DILI was associated with NAT2 slow acetylator genotype (odds ratio [OR], 3.18; 95% CI, 2.49–4.07) as well as GSTM1 null genotype (OR, 1.43; 95% CI, 1.08–1.88), amongst East Asians, Indian, Middle Eastern and Caucasians. In Asians, all three risk genes were significantly associated to risk of TB drug related DILI compared to Caucasians; CYP2E1*1A 1c/1c wildtype (OR, 1.35; 95% CI, 1.01–1.81), GSTM1 null genotype (OR, 1.55; 95% CI, 1.12–2.13), NAT2 slow acetylator (OR, 3.32; 95% CI, 2.43–4.53). These findings suggest that genetic factors involved in drug handling play an important role in TB drug related DILI susceptibility.

The strength of our study is that it provides for the first time frequencies and ranking of DILI comparing East and West, despite the limitations of the quality of the DILI reports, differences in disease prevalence and patterns of drug prescribing. Overall, the data syntheses show differences in DILI reporting and prevalence between East and West, both in drug class and individual agents. How should we use this information? The first clinical utility is that clinicians should be aware that certain classes and individual agents are more susceptible in the East to DILI, and monitor such patients more carefully, and the second is that certain classes of agents should be avoided (herbs and supplements) if they provide no clear benefit but come with increased risk, and finally drugs that are potentially hepatotoxic in the West, may not be so in the East. In the first instance, TB drug related DILI in the East has a 26.6% frequency amongst all DILI (but only 8.2% in the West, see Supplement Fig. 1-6) which makes these crucial agents to monitor for DILI. Liver failure due to TB drug related DILI is still a major but preventable problem that occurred in 14% of DILI cases in one report.11 This could be avoided if proper monitoring was in place with the knowledge that patients treated for TB have a high risk of DILI. The second important finding is that herbal DILI contributes 25.3% of DILI, and that many Asians are taking herbs and supplements without knowledge of their toxicity. One report of 177 cases acute liver failure from China, DILI accounted for 43.5%, and herbal DILI was the predominant cause in 39% of DILI cases.12 Patients should be forewarned that herbs and supplements pose a substantial risk without clear benefit. Lastly, certain drugs which have increased frequency of DILI in the West such as amoxicillin-clavulanate, is a rare cause of DILI in the East.

As discussed previously, limitations of our study are that the reported differences in prevalence of DILI may be confounded by the frequency of the disease in the countries concerned and consequently the frequency of the drugs prescribed, and not just explained by pharmacogenomics differences. It is beyond the scope of our study to examine such limitations, which can only be addressed by prospective collection of data in country specific databases. An example of differences in disease prevalence and incidence that could impact DILI reporting is that of TB. The World Health Organisation Tuberculosis Global Report,13 provides an overview of the incidence of TB and Asian countries contribute to 11 of the top 30 burden of disease countries with India (2,790,000) and Indonesia (1,020,000) contributing the largest burden. Overall Western countries (Americas, Eastern Mediterranean, and Europe) contribute to 1,330,000 cases but Asia (South-East Asia and Western Pacific) contributes 6,470,000 cases. In this setting, there are far more cases of TB in the East compared to the West and this may be a contributing reason for the high frequency of TB drug related DILI.

In conclusion, this study has shown clear differences in frequency of drugs implicated in DILI reported in Western studies and compared to Eastern studies, both by drug class (antibiotics are frequent DILI in the West while anti-TB drugs are frequent in the East), and for specific agents (Amoxicillin-clavulanate related DILI is the common in the West while INH_RIF_PZA is the common in the East). Knowledge of such differences and their pooled estimates of frequency, now provides clinicians of more precise dangers of DILI during prescribing, and to advise patients of potential
DILI when taking herbs and supplements. This field is also an area where research into genetic polymorphisms drug handling are much needed.

**Author’s contribution**

Overall concept and study design: SGL  
Search, retrieval and evaluation of articles: SGL, EXSL  
Analysis of quality and statistics: QZ, EC  
Manuscript draft and editing: SGL, EXSL, QZ, EC  
Final approval: SGL, EXSL, QZ, EC

**Conflicts of Interest**

The authors have no conflicts to disclose.

**SUPPLEMENTARY MATERIAL**

Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

**REFERENCES**

24. Galan MV, Potts JA, Silverman AL, Gordon SC. The burden of acute
Switching to systemic therapy after locoregional treatment failure: Definition and best timing

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In patients with unresectable hepatocellular carcinoma (HCC) without both macrovascular invasion and extrahepatic metastasis, the initial treatment choice recommended is transarterial chemoembolization (TACE). Before sorafenib came into wide use, TACE had been pointlessly carried out repeatedly. It was in the early 2010s that the concept of TACE refractory was advocated. Two retrospective studies from Japan indicated that conversion from TACE to sorafenib the day after patients were deemed as TACE refractory improved overall survival compared with continued TACE, according to the definition by the Japan Society of Hepatology. Nowadays, phase 3 trials have shown clinical benefits of several novel molecular target agents. Compared with the era of sorafenib, sequential treatments with these molecular target agents have gradually prolonged patients’ survival and have become major strategies in patients with HCC. Taking these together, conversion from TACE to systemic therapies at the time of TACE refractory, compared with before, may have a greater impact on survival and may be considered deeper in the decisions-making process in patients with unresectable HCC who are candidate for TACE. Up-to-date information on the concept of TACE refractory is summarized in this review. We believe that the survival of patients with unresectable HCC without both macrovascular invasion and extrahepatic metastasis may be dramatically improved by optimal timing of TACE refractory and switching to systemic therapies. (Clin Mol Hepatol 2020;26:155-162)

Keywords: Carcinoma, Hepatocellular; Liver neoplasms; Patient selection; Sorafenib

INTRODUCTION

Hepatocellular carcinoma (HCC) is the one of the worst malignancies, where mortality (781,631 deaths) closely parallels incidence (841,080 cases diagnosed).\textsuperscript{1,3} Frequent surveillance is recommended for the high-risk population of patients with HCC, such as those with cirrhosis or hepatitis virus infection, to diagnose the disease at an early stage.\textsuperscript{2,8} Patients with limited tumor...
burden with well liver function are the best candidates for resection. Another alternative treatment option for those with up to three nodules or nodules 3 cm or smaller (without both macrovascular invasion [MVI] and extrahepatic metastasis [EHM]) is local ablation therapy (radiofrequency ablation or microwave ablation).3–8 All over the world, the first-line treatment widely recommended for unresectable HCC without both MVI and EHM is transarterial chemoembolization (TACE), which is a locoregional treatment.3–8 Novel molecular target therapies have been proven the effectiveness in phase 3 studies, and thus, several treatment options have become standard systemic therapies for advanced-stage HCC or for those tumors progressing to locoregional treatment.9–14 However, creating a distinct definition of the timing of switching from locoregional treatment to systemic therapies in patients with HCC is not easy. Thus far, consultation with experts with advanced knowledge is needed when deciding to give up locoregional treatment and switching to systemic therapies in the field. Therefore, the aims of this study were to review the efficacy and limitations of TACE and discuss the most appropriate timing for switching from locoregional treatment to systemic therapies.

**RECONSIDERATION OF TACE FROM THE CURRENT PERSPECTIVE**

TACE is the most widely used treatment worldwide for unresectable HCC.2,3,15 The Bridge to Better Outcomes in HCC (BRIDGE) study, an international large and longitudinal cohort study that included 18,031 patients in 14 countries, indicated that TACE was frequently performed not only for patients with intermediate-stage HCC but also for patients with early- or advanced-stage HCC according to the Barcelona Clinic Liver Cancer (BCLC) staging system.15 Looking back at the history of TACE, improved patient survival with TACE compared with best supportive care or suboptimal treatments was demonstrated by a meta-analysis of six randomized controlled trials in 2003.16 Therefore, both Eastern and Western guidelines have recommended TACE as therapy in patients with intermediate-stage HCC (Table 1).4–8 So far, there are two major TACE techniques, namely, conventional TACE (cTACE), which uses a lipiodol mixture with chemotherapy agents, and TACE with drug-eluting bead (DEB-TACE).17–24 In one randomized controlled trial comparing cTACE and DEB-TACE, no difference was found in tumor response and time to progression.25 The decision whether to undergo cTACE or DEB-TACE seems to be dependent on the medical situation of each country or the institution.

Institutions with a high volume of patients or substantial experiences have advanced TACE techniques.20,25–28 So far, cone-beam or interventional radiology angiography computed tomography systems can construct three-dimensional reconstruction of arteries and support superselective TACE.29–31 Moreover, inserting a catheter into the tumor feeding arteries superselectively leads to the prevention of liver function and reduction in negative side effects. Using a large cohort of patients (n=815), Yamakado et al.32 reported that selective TACE contributed to survival outcomes in patients who received TACE (hazard ratio, 0.68; 95% confidence interval, 0.48–0.97; P=0.033). Another systemic review indicated that the most common adverse events were related to the postembolization syndrome, including increased liver enzyme (18.1%), fever (17.2%), abdominal pain (11.0%), vomiting (6.0%), and nausea (1.7%).20 In a recent randomized placebo-controlled trial,33 it was that indicated prophylactic dexamethasone prevented postembolization syndrome, although validation is required. Current treatment-related death rate is estimated to be less than 1% in patients with HCC.

**Table 1.** Recommendations for transarterial chemoembolization in guidelines all over the world

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian Pacific Association for the Study of the Liver (APASL) (2017)4</td>
<td>First line in patients with unresectable, large multifocal HCCs, without vascular invasion or extrahepatic spread.</td>
</tr>
<tr>
<td>European Association for the Study of the Liver (EASL) (2018)6</td>
<td>First line in BCLC B patients (not recommended for patients with decompensated liver disease, advanced liver and/or kidney dysfunction, macroscopic vascular invasion, or extrahepatic spread).</td>
</tr>
<tr>
<td>Korean Liver Cancer Association-National Cancer Center Korea (2019)7</td>
<td>Patients with a good performance status without major vascular invasion or extrahepatic spread who are ineligible for surgical resection and liver transplantation, RFA, or PEIT.</td>
</tr>
<tr>
<td>Japan Society of Hepatology (JSH) (2019)9</td>
<td>Unresectable Child-Pugh A and B patients with one to three nodules (≥3 cm) and more than four nodules (any size), without both vascular invasion and extrahepatic metastasis.</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; RFA, radiofrequency ablation; PEIT, percutaneous ethanol injection therapy.
A latest systemic review that had, to date, more than 10,000 patients with HCC undergoing TACE documented a 52.3% objective response, with overall 1-, 2-, 3-, and 5-year survival rates of 70.3%, 51.8%, 40.4%, and 32.4%, respectively. Nowadays, the median overall survival (OS) of patients with intermediate-stage HCC who received TACE is suggested to be around 30 months. In more than half of patients, objective response can be achieved by TACE, and tumor progression may be delayed in these patients. However, a latest international observation study (the outcomes of HCC patients treated with TACE and early, not early or not at all followed by sorafenib [OPTIMIS] study) found that with repeated TACE, expectation rates of objective response decreased (first TACE: 40%; second TACE: 26%; third TACE: 24%; and fourth TACE: 25%).

A recent report based on a US database (n=3,967) demonstrated deterioration of liver function from baseline in the acute period, which persisted in the chronic period (bilirubin, 30% and 23%; albumin, 52% and 31%; aspartate transaminase, 44% and 25%; alanine transaminase, 43% and 25%; and prothrombin time, 25% and 15%, respectively). Hiraoka et al., using a Japanese multicenter cohort, reported that liver function deteriorated with repeated TACE. It is well known that in patients with HCC, retaining liver function is equally important as removing or controlling tumor. That is, it is possible to experience decreasing liver function without getting an objective response when continuing TACE without appropriate decisions.

**WHAT IS “TACE REFRACTORY”?**

In most cases of unresectable HCC, before the use of sorafenib was approved, treatment with TACE was continued casually. Repeated TACE is associated with increased liver failure and diminishing efficacy. If treatment with TACE is continued without any assessment, the patient loses the opportunity to convert to systemic therapy because of liver function deterioration. Regarding recommendations or suggestions for patients with unresectable HCC to switch from TACE to systemic therapies, “TACE refractory” is a new-era concept that has been advocated since sorafenib was first used in practice (Table 2). It has been suggested that the appropriate timing of switching from TACE to systemic therapies is when repeated TACE is expected to become ineffective, the so-called TACE refractory. Kudo et al. from Japan, published one of the major suggestions on this topic. Their recom-

### Table 2. Recommendations/suggestions for switching from transarterial chemoembolization to systemic therapies in patients with unresectable hepatocellular carcinoma without both macrovascular invasion and extrahepatic metastasis

<table>
<thead>
<tr>
<th>Guidelines/articles</th>
<th>Recommendations/suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raoul et al. (2011)</td>
<td>Patients who progress after two cycles of TACE</td>
</tr>
<tr>
<td>JSH-LCSGJ criteria 2014 (so-called the definition of TACE failure/refractoriness) (2014)</td>
<td>(1) Intrahepatic lesion - Two or more consecutive insufficient responses of the treated tumor (viable lesion &gt;50%) even after changing the chemotherapeutic agents and/or reanalysis of the feeding artery seen on response evaluation CT/MRI at 1–3 months after having adequately performed selective TACE - Two or more consecutive progressions in the liver (tumor number increases as compared with tumor number before the previous TACE procedure) even after having changed the chemotherapeutic agents and/or reanalysis of the feeding artery seen on response evaluation CT/MRI at 1–3 months after having adequately performed selective TACE (2) Continuous elevation of tumor makers immediately after TACE even though slight transient decrease in observed (3) Appearance of vascular invasion (4) Appearance of extrahepatic spread</td>
</tr>
<tr>
<td>Asian Pacific Association for the Study of the Liver (APASL) (2017)</td>
<td>Treatment conversion from TACE to systemic therapy is recommended for patients in whom TACE is expected to be insufficient.</td>
</tr>
<tr>
<td>Galle et al. (2017)</td>
<td>Patients who have never or no longer respond to TACE (treatment stage migration to the right-hand side on the BCLC staging system).</td>
</tr>
<tr>
<td>American Association for Study of Liver Disease (AASLD) (2018)</td>
<td>Patients who are ineligible for or progress after TACE/TARE should be considered for systemic therapy.</td>
</tr>
<tr>
<td>Japan Society of Hepatology (JSH) (2019)</td>
<td>Molecular target therapy is recommended as second-line therapy for up to four intrahepatic nodules.</td>
</tr>
</tbody>
</table>

TACE, transarterial chemoembolization; CT, computed tomography; MRI, magnetic resonance imaging; BCLC, Barcelona Clinic Liver Cancer; TARE, transarterial radioembolization.
mendation was validated in two retrospective studies that showed that patients with intermediate-stage HCC whose disease was refractory to TACE and who switched to sorafenib had prolonged OS compared with those who continued TACE (first trial: 25.4 vs. 11.5 months, \( P = 0.003 \); second trial: 24.7 vs. 13.6 months, \( P = 0.002 \)).\(^{42,43}\) Currently, a large cohort global prospective observation study (OPTIMIS) confirmed that compared with other treatments, switching to sorafenib immediately after TACE ineligibility improved OS (propensity score matched cohort: 15.2 vs. 11.8 months).\(^{34}\)

Regarding liver function, one Japanese study confirmed that liver function was not preserved with continuing TACE treatment after TACE refractory; patients with TACE refractory who continued to receive TACE had a significantly shorter median time to liver dysfunction than did those who switched to sorafenib (17.0 vs. 29.8 months, \( P = 0.030 \)).\(^{42}\) In another study, patients with TACE refractory who underwent repeated TACE had a greater increase in Child-Pugh score compared with patients who were converted to sorafenib.\(^{43}\) Taken together, these two studies strongly suggest that repeated TACE leads to deterioration in liver function in patients who are deemed refractory to TACE.

**WHY IS “TACE REFRACTORY” EMPHASIZED NOW?**

At present, phase 3 trials have proven the statistical significance of five molecular target agents as both first-line (sorafenib and lenvatinib) and second-line (regorafenib, ramcprumab, and cabozantinib) treatments for patients with HCC.\(^{9-14}\) These drugs have been approved worldwide. Moreover, on the basis of early-phase trials, the US Food and Drug Administration also approved two immune checkpoint inhibitors (nivolumab and pembrolizumab).\(^{44,45}\) Looking back at the results of the phase 3 trials in patients with HCC, both the testing and control arms had increased median OSs during the previous decade as a result of the treatments after progression.\(^{9,10,14,46,47}\) In the study of regorafenib after sorafenib in patients with HCC (RESORCE) trial, Finn et al.\(^{48}\) reported a median OS of 26.0 months for the regorafenib arm from the time sorafenib was started, although the included population in the trial was limited because of strict inclusion criteria. Several retrospective studies have reported that sequential therapies might have an impact on the survival of patients with advanced HCC.\(^{49,50}\) In the next era, a major strategy in patients with HCC seems to be sequential treatment with systemic therapies, although different countries or regions have different regulations regarding the use of each drug. That being said, in TACE refractory patients, survival outcomes with systemic therapies are suggested to be longer than with treatment with a single agent, like sorafenib.

Moreover, when considering using two or more molecular target agents in the clinical treatment of identical patients with HCC, we should focus on maintaining liver function more than we did in the previous era. Liver function deterioration leads to missed opportunities to start or switch to systemic therapies. Several latest reports have demonstrated that the high-tumor-burden population of patients with intermediate-stage HCC (large size or/and a lot of nodules) were at high risk of liver function deterioration after TACE.\(^{51-53}\) Two retrospective studies, which criticized the Japan Society of Hepatology’s definition of TACE refractory, reported that 20–25% of patients had decreased liver function and Child-Pugh class B or C at the time they were deemed TACE re-

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**Figure 1.** Concept of “Stop and think carefully before proceeding to each TACE.” TACE, transarterial chemoeembolization.

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\[\text{https://doi.org/10.3350/cmh.2019.0021n}\]

\[\text{http://www.e-cmh.org}\]
fractory even though they were determined to have Child-Pugh class A at the time of initial TACE.\textsuperscript{42,43} Taken together, the optimal timing to convert from TACE to systemic therapies may be when the patient’s condition becomes unstable, which is different among individuals based on each patient’s tumor status, treatment outcome of previous TACE and hepatic spare ability.

Recently, Kudo et al.\textsuperscript{54} compared survival outcomes between TACE and lenvatinib in seven patients with intermediate-stage HCC by using the propensity score matching method. In this study, the lenvatinib group had no history of receiving TACE. Despite the presence of several biases, the result might suggest the possibility that systemic therapies can become the initial treatment choice and may replace TACE in a limited population of patients with intermediate-stage HCC. A randomized controlled trial that compares systemic therapy and TACE will be carried out in the near future.

STOP AND THINK CAREFULLY BEFORE PROCEEDING TO EACH TACE

With the development of novel compounds and the improving survival outcomes of patients with HCC, current systemic therapies should be considered as a treatment option for unresectable HCC. Especially in patients with intermediate-stage HCC, who can expect long clinical courses with better prognosis compared with advanced-stage HCC, updated treatment strategies with molecular targets focusing on not only short-term outcomes but also foresight with long-term perspectives are required. A validity assessment of the TACE procedure should be recommended before proceeding to each TACE (Fig. 1). In cases where the effectiveness of TACE is not expected or chronic liver function deterioration may occur after TACE, conversion to systemic therapies should be considered. Although the definition of TACE refractory by the Japan Society of Hepatology is useful information, switching the timing of TACE to way before the patient’s liver function deteriorates, taking into account both the high expectations of clinical outcome with current sequential therapies of molecular target agents and preventing hepatic spare abilities, may be better. Radiologists, who have the responsibility for TACE, and hepatologists or oncologists should communicate more closely and construct treatment strategies for patients with unresectable HCC who receive TACE with a view of the prospect to convert from TACE to systemic therapies.

CONCLUSIONS

The present guidelines recommend TACE as first-line treatment for patients with unresectable HCC without both MVI and EHM. Several novel compounds have shown survival benefits in patients with advanced HCC. Nowadays, sequential therapies with two or more agents have prolonged patients’ survival outcomes and have become predominant treatment strategies among patients with HCC. The anticipated clinical impact of systemic therapies in patients with HCC has increased, and conversion from TACE to systemic therapies is now weighed more heavily. Before starting treatment with TACE, conversion to systemic therapies or not should be considered (“Stop and think carefully before proceeding to each TACE”). Looking ahead to the future of systemic therapies, it is now necessary to foster a greater understanding of preserving liver function during TACE treatments.

Authors’ contribution

SS, YO, Keisuke Koroki, SM, HK, Kengo Kanayama, Kazufumi Kobayashi, SK, MN, Naoya Kanogawa, TS, TK, ES, SN, AT, TC, MA, JK designed the article. SS and YO took the lead in writing the manuscript. Naoya Kato supervised the manuscript. All authors provided critical feedback and approved the manuscript.

Acknowledgements

We would also like to thank Enago (www.enago.jp) for the English language review.

Conflicts of Interest

Sadahisa Ogasawara received honoraria from Bayer, Eisai, Eli Lilly, consulting or advisory fees from Bayer, Eisai, Merck & Co., Inc., Chugai Pharma, Eli Lilly, AstraZeneca, and research grants from Bayer and Eisai. Yoshihiko Ooka received honoraria from Eisai. Naoya Kato received honoraria from Bayer and Eisai, consulting or advisory role from Bayer and Eisai, research funding from Bayer and Eisai. The other authors who took part in this study indicated that they did not have anything to declare regarding funding or conflict of interest with respect to this study.

REFERENCES


29. Pua U, Teo CC, U PT, Quek LHH. Cone-beam CT acquisition during transradial TACE made easy; use of the swivel arm board. Br J Radiol 2018;91:20170248.


Regulated differentiation of stem cells into an artificial 3D liver as a transplantable source

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End-stage liver disease is one of the leading causes of death around the world. Since insufficient sources of transplantable liver and possible immune rejection severely hinder the wide application of conventional liver transplantation therapy, artificial three-dimensional (3D) liver culture and assembly from stem cells have become a new hope for patients with end-stage liver diseases, such as cirrhosis and liver cancer. However, the induced differentiation of single-layer or 3D-structured hepatocytes from stem cells cannot physiologically support essential liver functions due to the lack of formation of blood vessels, immune regulation, storage of vitamins, and other vital hepatic activities. Thus, there is emerging evidence showing that 3D organogenesis of artificial vascularized liver tissue from combined hepatic cell types derived from differentiated stem cells is practical for the treatment of end-stage liver diseases. The optimization of novel biomaterials, such as decellularized matrices and natural macromolecules, also strongly supports the organogenesis of 3D tissue with the desired complex structure. This review summarizes new research updates on novel differentiation protocols of stem cell-derived major hepatic cell types and the application of new supportive biomaterials. Future biological and clinical challenges of this concept are also discussed. (Clin Mol Hepatol 2020;26:163-179)

Keywords: Stem cells; Differentiation; Decellularized matrix; Natural macromolecules; Synthetic polymers

INTRODUCTION

In the clinical context, chronic liver disease is a process in which the liver undergoes progressive destruction and regeneration, which lead to fibrosis and cirrhosis. It is one of the major causes of mortality worldwide. In 2016, chronic liver disease and cirrhosis caused a total of 40,545 deaths, in which alcohol-related liver disease induced nearly half of the deaths.1 Since damage in the liver often induces inflammation, oxidative stress, cellular necrosis and apoptosis, which causes tissue scarring and fibrosis if occur-
ring repeatedly, developing therapies that can retard or reverse those pathological processes is quite important. When liver disease progresses to the end stage, it becomes irreversible and liver failure is inevitable. At this point, liver transplantation is the only clinical option. However, the lack of transplantable livers and the possibility of immune rejection after the transplant seriously hinders its wide application. In addition, the maximal cold preservation duration of the human liver organ soaked in University of Wisconsin solution before a clinical transplantation operation is only 6-12 hours. Developing novel supercooling techniques to extend this duration to 24-36 hours will dramatically enlarge the donation territory, as well as reduce logistical pressures and facilitate recipient preparation.

To solve the problems hindering successful clinical liver transplants, stem cell medicine provides a brand new solution, particularly those stem cells with the potential to differentiate into liver cells that can then be transplanted directly. For example, a large number of studies have proved that the transplantation of differentiated hepatocytes from human adipose derived stem cells, human amniotic epithelial stem cells, and inducible pluripotent stem cells (iPSCs) effectively ameliorated the complications induced by acute liver failure, including elevated serum aminotransferases, hepatic inflammation, and cell death. Stem cell therapy was also reported to be successful in other kinds of chronic/end-stage liver diseases, including nonalcoholic steatohepatitis, Wilson’s disease, cirrhosis, and hepatocellular carcinoma. However, mechanistic studies found that the life support and hepatic protective effects of exogenous stem cells, including mesenchymal stem cells (MSCs) and iPSCs, were primarily from their paracrine actions instead of from direct hepatic occupation. Only hepatic progenitor cells were able to completely and functionally reconstitute a liver that had suffered otherwise lethal damage.

Although strategies with differentiated hepatocytes and naïve stem cells demonstrated massive success in the past decades, single cell type-based injection or “in situ transplantation” cannot meet the majority of clinical requirements, which need physiologically functional hepatic tissue to ameliorate damage and support normal liver functions within a short duration. To solve this problem, scientists began to use a complicated but fascinating method – constructing artificial organs from stem cells in three-dimensional (3D) culture. In the current review, we focus on the research updates on liver organogenesis from stem cells, with emphases on the differentiation protocol, biomaterial support, and self-condensation mechanisms.

3D ORGANOGENESIS FROM STEM CELLS

Over the past decade, significant progress has been made in controlling cellular differentiation in stem cell research. For example, it is now possible to force MSCs and iPSCs to differentiate into a large number of specific somatic cell lineages by mimicking the signals presented during embryogenesis. Very importantly, several studies have demonstrated that stem cells have the ability to self-organize into a functional tissue by scattering various somatic cells throughout the tissue. For example, Takebe et al. generated a vascularized and functional human liver from human iPSCs by transplanting liver buds created in vitro. Such liver buds were created from a mixture of human iPSCs, human MSCs, and human umbilical vein endothelial cells (HUVECs). The cells then underwent self-organization into a 3D liver bud by recapitulating the organogenetic interactions between the endothelial and mesenchymal cells. The authors tested the hepatic functions of the liver buds both in vitro and in a murine liver failure model, which exhibited satisfactory outcomes. They then expanded this strategy to even more vascularized artificial organs, including kidney, pancreas, intestines, heart, lungs, and brain. It was found that mesenchyme-driven self-condensation on a soft matrix is crucial for organ bud generation. Although those in vitro-grown complex tissue architectures have limited size and survival duration, the ideas raised by this Japanese group extensively inspired other regenerative medicine researchers and clinicians to consider that a mixture of hepatic cell types that are necessary for organ development and self-condensation (e.g., hepatocytes, stellate cells, sinusoidal endothelial cells, and bile duct epithelial cells), together with guides from undifferentiated stem cells, is a promising strategy to generate a clinically transplantable liver or other organ.

DIFFERENTIATION INTO HEPATIC CELL TYPES

Hepatocytes

Hepatocytes are the major cell type of the liver and account for approximately 80% of the hepatic volume and quantity. They are a highly differentiated parenchymal cell type. There are more than 2.5 billion hepatocytes in an adult human liver, and a hepatic lobule is made up of 5 million hepatocytes. Active hepatocyte metabolism can synthesize human essential blood coagulation factors, aliphatic acid, cholesterol, and phospholipids; hepatocytes
Hepatic stellate cells (HSCs)

HSCs are the major nonparenchymal cells of the liver. Under physiological conditions, HSCs are in a quiescent state in which their main function is to store and transport vitamin A. When the liver is damaged, under the actions of inflammatory cytokines and stress factors, HSCs change into an activated state (a myofibroblastic phenotype) characterized by increased proliferation, contractility, and chemotaxis. The activation of HSCs will promote the secretion of the ECM involved in liver injury repair. Thus, HSCs play an important role in the occurrence and development of various liver diseases. Moreover, through their interaction with other liver cell types, HSCs are also involved in liver regeneration and differentiation.

Research on HSC formation from stem cells is scarce. The main reason for this is that the embryonic origin of HSCs is yet unresolved, with hypotheses of mesenchymal and endodermal origins. Baba et al. proved that in a murine model, HSCs are from the bone marrow since they administered bone marrow cells from green fluorescent protein (GFP) transgenic mice to age-matched mice, and found GFP-positive HSCs in the recipient livers. However, an analytical study of cell lineage demonstrated that HSCs are derived from the mesoderm during liver development; in particular, they are derived from the mesothelium (consisting of mesothelial cells and submesothelial cells) which migrate inward from the liver surface to form HSCs and perivascular mesenchymal cells. Other studies claimed that HSCs are important for the association with hematopoietic sites in the fetal rat liver. HSCs can fulfill some of the functions of MSCs, such as adipocytes or osteocytes differentiation. Thus far, only Coll et al. reported that they could obtain HSC-like cells from newborn male fibroblasts-derived human iPSC by mimicking embryonic development; those cells closely resemble primary human HSCs at the transcriptional, cellular, and functional levels and possess a gene expression profile between that of quiescent and activated HSCs.

Liver sinusoidal endothelial cells (LSECs)

LSECs consist of the majority of liver nonparenchymal cells (~70% of the total liver nonparenchymal cells). They are functionally unique because of the high activity of receptor-mediated endocytosis, which enables them to clear colloids and soluble waste macromolecules from circulation. LSECs have long been noted to contribute to liver regeneration after liver injury. That is, there is crosstalk between HSCs and LSECs when the activation of HSCs are also able to store glycogen, proteins, lipids, and vitamins to fulfill the body's needs.

In the liver, hepatocytes are the very basic cells that support its essential physiological functions. Over the past decade, several studies have introduced protocols to induce hepatocyte-differentiation in stem cells; most methods use chemical and conditioned medium stimulations or coculture with other cell types to expedite hepatic differentiation from human bone marrow mesenchymal stem cells, human umbilical cord mesenchymal stem cells, and human iPSCs. Induced hepatocyte-like cells (i-Hep) are shown to possess most of the normal hepatocyte functions (including the secretion of aminotransferases, albumin, and α-fetoprotein), functional biotransformation systems, and the ability to engraft, integrate, and proliferate in an injured liver. Nie et al. used human iPSC to generate a functional liver organoid, which exhibited stronger hepatic functions than human iPSC-derived hepatocyte-like cells (or i-Hep). They found that the functional liver organoid was more susceptible to hepatitis B virus (HBV) infection and could maintain HBV propagation and produce the infectious virus for a prolonged duration, thus causing the hepatic dysfunction of the liver organoid via the down-regulation of hepatic gene expression, induced release of early acute liver failure markers, and altered hepatic ultrastructure. The liver organoid may provide a promising individualized infection model for the development of individualized treatment for hepatitis. Different stem cell types require different concentrations of inducing reagents, including hepatocyte growth factor (HGF), fibroblast growth factor (FGF), oncostatin M (OSM), epidermal growth factor (EGF), and bone morphogenetic protein. Currently, one can even conveniently purchase a commercial one-step direct hepatocyte induction kit.

Although still in the primary stages, a number of studies have tried to induce and culture differentiated hepatocytes in a 3D manner in vitro. The primary interest in developing 3D-structured hepatocytes is because although the 2D in vitro culture of hepatocytes is currently mature, 2D-cultures show a reduction in major liver functions, such as a decreased secretion of albumin and impaired phase I and II enzymatic detoxification abilities. The application of extracellular matrix (ECM) is a major solution for these problems. Thus far, the most common strategy is the sandwich structure in which hepatocytes are placed between two layers of ECM. This model has been proven to provide better hepatocyte cellular functions than 2D monolayer culture conditions since it promotes a polygonal hepatocyte morphology and extended contact surfaces between the cells and the matrix.
and the formation of fibrosis occur. Healthy LSECs are able to suppress the activation of HSCs and also inactivate already-activated HSCs. Conversely, in the healthy liver, hepatocytes and HSCs can work together to maintain the homeostasis of LSECs through the release of vascular endothelial growth factor (VEGF). When the Kupffer cells (KCs)’ phagocytic function is damaged, LSECs will remove materials with diameters greater than 1 μm. Furthermore, LSECs take in viruses and submit antigens to T cells. Additionally, they participate in the local immune regulation in the liver and may also play an important role in immune tolerance.

Reports of direct differentiation from stem cells to LSECs are extremely limited. Du et al. demonstrated a novel two-step method for that process, that is, a 7-day first stage from human iPSCs that are generated by reprogramming human foreskin fibroblasts to the mesodermal lineage and a 7-day second stage from mesodermal cells to hepatic endothelial cells. The final LSEC-like cells are positive for expressions of endothelial-specific markers such as CD-31 and VE-cadherin. claimed that they established a culture condition to promote the differentiation from multiple iPSC cell lines to endothelial cells that coexpress cell surface markers and functional properties (e.g., endothelial nitric oxide synthase production) with an efficiency of approximately 10–40%. In addition, Narmada et al. declared that they had formed endothelial cells from human iPSC that had been derived from IMR90 fibroblasts and BJ fibroblasts by using a chemically defined hepatocyte growth medium (HGM); those cells expressed PECAM-1 and CDH5, could form spontaneous tube structures that represent mature endothelial markers, possessed endothelial nitric oxide synthase, and were capable of taking up acetylated low-density lipoprotein.

**Bile duct epithelial cells (BDECs)**

BDECs are a kind of distinctive epithelial cell coating on the liver inner tube surface. They are of great importance in bile duct contraction, bile secretion, and liver water electrolyte transport. In addition, BDECs can also mediate the synthesis and secretion of IgA/IgM, cytokines, chemokines, and adhesion molecules involved in immunity, cell interaction, and signal transduction.

A study described a differentiation procedure from embryonic stem cells (ESCs) to BDECs on the basis of induced hepatocytes. The authors coincubated the ESCs with HGF/scatter factor, EGF, and transforming growth factor α (TGF-α) in the presence of a new HGM. In addition to the expected morphology, the differentiated BDECs also expressed cytokeratin 19, acidic FGF, and TGF-α, but did not express the hepatocyte markers including albumin and cytochrome P450 1B1. Another study confirmed that dexamethasone and transforming growth factor β (TGF-β) contribute to differentiation into BDECs; these compounds are closely connected with the Wnt/β-catenin associated protein, and the Notch and TGF-β signaling transduction pathways.

Sampaziotis et al. from the University of Cambridge reported a novel and serum-free method for the direct differentiation of cholangiocytes from human skin fibroblasts and peripheral blood derived iPSCs. By using several combinations of recombinant growth factors and inhibitors at different stages, they formed definitive endoderm, foregut progenitors, hepatoblasts, and cholangiocyte progenitors from iPSCs in sequence. After a 26-day differentiation process, the final cholangiocyte-like cells were capable of showing the functional characteristics of normal cholangiocytes, including alkaline phosphatase/γ-glutamyltranspeptidase activity, transfer of bile acids, and responses to secretin, somatostatin, and VEGF. The derived cells were also useful in establishing some common biliary disease models (e.g., Alagille syndrome, polycystic liver disease, and cystic fibrosis-associated cholangiopathy).

**Kupffer cells (KCs)**

KCs are hepatic local macrophages which are located on the inner surface of the sinus hepaticus. They are a critical component of the mononuclear phagocytic system, which takes part in the response to pathogens. KCs can remove foreign antigens, antigen-antibody complexes, cell debris, and other substances in the liver and circulation partly through the activation of nuclear factor kappab and the secretion of a variety of cytokines. The abnormal activation and regulation of KCs contribute to nearly all types of liver diseases, such as acute liver failure, nonalcoholic fatty liver disease, alcohol-related liver disease, genetic liver diseases, cirrhosis, and liver cancer.

Although a number of studies have successfully established methods to generate macrophage or dendritic cell from human PSCs, no report described the differentiation protocol from stem cells to KCs until 2018. Part of the reason is that the derived macrophage is able to migrate into the liver to form KCs and pit cells (intrahepatic lymphocytes). In addition, in the process of establishing an artificial 3D liver, KCs and pit cells are not necessary because 1) they are expected to migrate into the engineered liver grafts from the bone marrow and the circulatory system of the recipients upon transplantation, 2) the functions of the assembled
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<td>Hepatocytes</td>
<td>ESCs</td>
<td>Human 1st stage: RPMI+B27 with activin and NaB for 5 days; 2nd stage: Knockout DMEM + 20% SR with DMSO for 7 days; 3rd stage: CL15 with HGF and oncostatin M for 7 days</td>
<td>Expression of HNF4α, ALB, FoxA2, TTR, A1AT, and AFP</td>
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<td>MSCs</td>
<td>Human 1st stage: DMEM/F-12 with HGF, EGF, ITS and dexamethasone for 2 weeks; 2nd stage: DMEM/F-12 with HGF, EGF, ITS, dexamethasone and oncostatin M for 2 weeks</td>
<td>Expression of CK-18, ALB and AFP</td>
<td>6</td>
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<td>ESCs and iPSCs</td>
<td>Human 1st stage: RPMI+B27 with activin A for 5 days; 2nd stage: RPMI+B27 with BMP-4 and FGF2 for 5 days (20% CO2); 3rd stage: RPMI+B27 with BMP-4 and FGF2 for 5 days; 4th stage: RPMI+B27 with HGF; 5th stage: HCM with oncostatin M</td>
<td>Secretion of albumin, expression of FOXA2, HNF4α and AFP</td>
<td>110</td>
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<td></td>
<td>ESCs and iPSCs</td>
<td>Human 1st stage: RPMI+B27 with CHIR99021 for 1 day and removal of CHIR for another day; 2nd stage: KOSR with DMSO for 5 days; 3rd stage: L15 with Dihexa and dexamethasone for 10 days</td>
<td>Expression of albumin, HNF4α, AAT and CYP3A4</td>
<td>111</td>
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<td>ADSCs</td>
<td>Human 1st stage: 10% FBS RPMI-1640 with ATRA for 1 day; 2nd stage: serum-free RPMI-1640 with IDE1, CHIR99021, LY294002 for 1 day; 3rd stage: serum-free RPMI-1640 with IDE1, LY294002, LDN-193189, FGF4 for 2 days; 4th stage: serum-free RPMI-1640 with IDE1, LY294002, FGF4 for 1 day; 5th stage: Williams’ E with HGF, FGF4, oncostatin M, dexamethasone, ITS for 5 days</td>
<td>Expression of HNF4α, AAT, ALB, TDO2, AFP, CYP3A4, TTR, CK-18. With capacity for glycogen storage.</td>
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<td>PDLSCs</td>
<td>Human 1st stage: 2% FBS Knockout DMEM with HGF for 5 days; 2nd stage: Knockout DMEM with oncostatin M, dexamethasone, ITS for 16 days</td>
<td>Expression of HNF4q, CK-18, ALB, TAT, TDO, AAT and presence of glycogen storage</td>
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<td></td>
<td>ESCs and iPSCs</td>
<td>Human 1st stage: mTeSR™ medium with DMSO for 1 day; 2nd stage: RPMI 1640 + B27 with CHIR99021 for 1 day; 3rd stage: RPMI 1640 + B27 alone for 1 day; 4th stage: advance F12 with A83-01, sodium butyrate, DMSO for 5 days; 5th stage: advance F12 with FH1, FPH1, A83-01, dexamethasone, hydrocortisone for 5 days</td>
<td>Expression of ALB, AAT, TTR, APOA2, HNF4α, and CYP450 enzymes. Secretion of albumin and capacity for glycogen storage.</td>
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<td>iPSCs</td>
<td>Porcine 1st stage: RPMI with activin A, Wnt 3a for 1 day; 2nd stage: SF6 with activin A, bFGF for 5 days; 3rd stage: SF6 with bFGF, BMP4, EGF, HGF for 3 days; 4th stage: SF6 with γ-secretase inhibitor-X, HGF, OSM, DMSO for 3 days; 5th stage: SF6 with HGF, OSM, dexamethasone for 6 days</td>
<td>Expression of ALB, HNF1α, CK-18, TFR, CRK-8, AFP. Expression of metabolizing phase I phase II enzymes, and phase III transporters.</td>
<td>25</td>
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</table>

**Hepatic stellate cells**

| iPSCs | Human | 1st stage: DMEM with Wnt3a and activin-A for 6 days; 2nd stage: DMEM with bFGF and BMP4 for 4 days; 3rd stage: DMEM with aFGF, FGF4 and FGF8b for 4 days; 4th stage: DMEM with HGF, dexamethasone, and follistatin for 7 days | Vitamin A storage, expression of GPR91, ALCAM, and CRBP | US patent 20120009672 A1 |
| iPSCs | Human | DMEM low glucose with MCD-201-water, linoleic acid-bovine serum albumin, insulin-transferrin-selenium, penicillin streptomycin, L-ascorbic acid, dexamethasone, 2-mecaprotoethanol. Growth factors were added as follows: BMP4 from day 0 to day 4, FGF1 and FGF3 from day 4 to day 8, retinol and palmitic acid from day 6 to day 12 | Expression of DES, ALCAM, GFAP, SYP, ACTA2, PDGFβ, LRAT, COL1α1, RELN, PCDH7 | 35 |
Table 1. Continued

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<th>Liver sinusoidal endothelial cells</th>
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<td>iPSCs</td>
<td>Human</td>
<td>1st stage: RPMI1640 with ITS A, non-essential amino acids, l-glutamine, 2-mercaptoethanol, activin-A, BMP-4, bFGF, rm-Wnt-3a, and SB431542 for 7 days; 2nd stage: EndoGro media with VEGF and rh-bFGF for 7 days</td>
<td>VE-cadherin and PECAM1</td>
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<td>ESCs and iPSCs</td>
<td>Human</td>
<td>1st stage: mTeSR1 with FGF2, LY294002, BMP4 for 1.5 days; 2nd stage: mTeSR1 with FGF2, BMP4 for 3.5 days; 3rd stage: mTeSR1 with FGF2, SB431542, VEGF for 5 days; 4th stage: commercial medium EGM-2</td>
<td>Expression of PECAM 1, CDH5, eNOS. With capacity for taking up acetylated LDL and forming tubes.</td>
<td>42</td>
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<th>Bile duct epithelial cells or cholangiocytes</th>
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<td>Primary hepatocyte</td>
<td>Human and rat</td>
<td>HGM medium with HGF, EGF. After 68 hours, the media was replaced with the supernatant from CRgP-packaged, replication deficient, amphotropic retrovirus containing the E. coli β-galactosidase gene under an LTR promoter. Polybrene was added at 2 μg/mL.</td>
<td>CK-19, production of TGF-α and acidic FGF</td>
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<td>iPSCs</td>
<td>Human</td>
<td>1st stage: CDM with activin-A, FGF2, BMP4, LY294002, CDM for 3 days; 2nd stage: RPMI with activin-A for 5 days; 3rd stage: RPMI with SB and BMP4 for 4 days; 4th stage: RPMI with FGF10, RA and activin-A for 4 days; 5th stage: William E with EGF on Matrigel 3D culture for 10 days</td>
<td>Bile acids transfer, AP and GGT activity and responses to secretin, somatostatin and VEGF</td>
<td>46,47</td>
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<th>Kupffer cells</th>
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<tr>
<td>iPSCs</td>
<td>Human</td>
<td>1st stage: mTeSR™ with BMP-4, VEGF, SCF, ROCK inhibitor for 12 days; 2nd stage: X-VIVO™ media with M-CSF, IL-3, glutamin, β-mercaptoethanol for 6 days; 3rd stage: Primary hepatocyte conditioned media and advanced DME plus supplements for 5–7 days</td>
<td>Expression of CD14, CD11, CD32, CD68, CD163, production of TNF-α and IL-6</td>
<td>55</td>
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ESCs, embryonic stem cells; RPMI, Roswell Park Memorial Institute medium; NaB, sodium butyrate; SR, knockout serum replacement; DMEM, Dulbecco’s modified Eagle medium; DMSO, dimethyl sulfoxide; HGF, hepatocyte growth factor; ALB, albumin; FoxA2, forkhead box protein A2; TTR, transthyretin; A1AT, α1-antitrypsin; AFP, α-fetoprotein; MSCs, mesenchymal stem cells; EGF, epidermal growth factor; ITS, insulin-transferrin-selenium; CK, cytokeratin; iPSCs, induced pluripotent stem cells; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; HCM, hepatocyte culture medium; HNF, hepatocyte nuclear factor; KOSR, knockout serum replacement; AAT, α1-antitrypsin; VEGF, vascular endothelial growth factor; CK-19, production of TGF-α and acidic FGF; VEGF, vascular endothelial growth factor; ACTA2, alpha 2 smooth muscle actin; DMSO, dimethyl sulfoxide; HGF, hepatocyte growth factor; SCF, stem cell factor; ROCK, Rho-associated kinase; M-CSF, macrophage colony stimulating factor; IL, interleukin.

LSECs are similar to those of KCs, and KCs may secrete nitric oxide to increase their own apoptosis, leading to acute rejection after liver transplantation. Additionally, KCs will induce apoptosis and differentiation of T-cells through the Fas-FasL apoptosis pathway. Thus far, only Tasnim et al. demonstrated that they could obtain mature KCs from human IMR90 fibroblasts derived iPSCs (iKCs). By using a different culture medium and several combinations of recombinant growth factors and inhibitors at different stages, they generated KCs that expressed the macrophage markers CD11, CD14, CD68, CD163, CD32 at 0.3−5 folds of those from...
primary adult human KCs (pKCs) and KC-specific CLEC-4F, ID1, and ID3. Moreover, iKCs phagocytosed and secreted IL-6 and TNF-α upon stimulation at levels similar to pKCs but different from nonliver macrophages.\textsuperscript{55} Although not necessary for 3D liver assembly, KCs should be included in the 3D liver for disease modeling and drug discovery \textit{in vitro}. The reported differentiation protocols of the above-mentioned hepatic cell types are listed in Table 1. Key markers for differentiated liver cell types were summarized in Figure 1.

**ASSEMBLY OF TRANSPLANTABLE 3D LIVER TISSUE**

In previous decades, numerous studies have focused on the construction of liver tissue in a 2D planar environment. However, such a strategy is challenging because the actual liver tissue is 3D. Therefore, to address the limitations of 2D cell cultures and achieve the functional recovery of the liver, a 3D microenvironment which aims to mimic the native environment must be fabricated.\textsuperscript{56} Recently, scientists focused on exploring artificial 3D ECM by fabricating a scaffold using particular materials or a matrix, including a decellularized matrix, natural macromolecules, and synthetic biodegradable polymers (listed in Table 2).\textsuperscript{57} The engineered scaffolds can provide a 3D environment for cell attachment and proliferation. Moreover, the scaffolds play a role in regulating cell maturation and function by modification with specific physical and biochemical properties.\textsuperscript{58,59} Recently, by transdifferentiating hepatocytes, HSCs, and LSECs from human umbilical cord blood stem cells simultaneously, we obtained a self-assembled functional 3D human liver bud \textit{in vitro} and transplanted it into a murine model of acute liver failure. The liver bud underwent maturation during

**Figure 1.** Key functional markers of liver cell types differentiated from stem cells (e.g., embryonic stem cells, mesenchymal stem cells, and inducible pluripotent stem cells). Major liver cell types, including hepatocytes, Kupffer cells, hepatic stellate cells, liver sinusoidal endothelial cells, and cholangiocytes can be reprogrammed from various stem cells using defined medium and chemical compounds during in vitro culture. Key functional markers can be used to validate the successful reprogramming of each cell type. ALB, albumin; HNF4α, hepatocyte nuclear factor 4 α; CK, cytochrome; AFP, α-fetoprotein; AAT, α-1 antitrypsin; CYP, cytochrome P family enzymes; PECAM, platelet endothelial cell adhesion molecule-1; eNOS, endothelial nitric oxide synthase; ESCs, embryonic stem cells; MSCs, mesenchymal stem cells; iPSCs, inducible pluripotent stem cells; ALCAM, activated leukocyte cell adhesion molecule; CRBP, cellular retinol-binding protein; COL1α1, collagen type 1 α 1; GFAP, glial fibrillary acidic protein; RELN, reelin; PCDH7, protocadherin-7; aFGF, acidic fibroblast growth factor.
### Table 2. Novel biomaterials for 3D culture and assembly of stem cell-derived liver cells

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<td>Rat primary hepatocyte</td>
<td>Porcine liver-derived biomatrix</td>
<td><em>In vitro</em> culturing with biomatrix for up to 45 days</td>
<td>Better albumin synthesis, urea production, and P450 IA1 activity</td>
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<td>Rat hepatocyte</td>
<td>Decellularized liver</td>
<td>Transplant of recellularized liver grafts into rats</td>
<td>Enhanced liver-specific functions and survival with minimal ischemic damage</td>
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<td>Rat BMSCs</td>
<td>Decellularized spleen scaffold</td>
<td><em>In vitro</em> culturing within the 3D matrix</td>
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<td>Human MSC</td>
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<td><em>In vitro</em> culturing with sequential chemically induced protocol</td>
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<td>Human MSC</td>
<td>2D collagen films and 3D collagen scaffolds</td>
<td><em>In vitro</em> 2D and 3D culture for 21 days</td>
<td>Significant increase in claudin expression compared to conventional monolayer culture and 2D collagen scaffold</td>
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<td>Human MSC</td>
<td>3D matrigel/collagen scaffolds</td>
<td><em>In vitro</em> 3D matrigel/collagen scaffolds cocultured with HUVECs in the presence of fetal liver extract for 14 days</td>
<td>Induced the cell expression of some early liver-specific markers</td>
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<td>Human iPSC</td>
<td>Collagen vitrigel membrane</td>
<td><em>In vitro</em> culturing on collagen vitrigel membrane for 30–40 days</td>
<td>Increased the expression of mature hepatocyte transcription factors and mature markers involved in liver functions</td>
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<td>Human ADSCs</td>
<td>3D gelatin scaffolds</td>
<td><em>In vitro</em> culturing on 3D gelatin scaffold more than 2 weeks</td>
<td>Promoted the differentiation to hepatocyte-like cells with higher expression of specific markers and levels of urea biosynthesis and glycogen storage</td>
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<td>Human ADSCs</td>
<td>Gelatin-laminin cryogel scaffolds</td>
<td><em>In vitro</em> culturing for 25 days</td>
<td>Significantly promoted differentiation and the resulting cells were strikingly similar to HepG2 in terms of expressing hepatocyte markers</td>
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<tr>
<td>Rat hepatocyte</td>
<td>Chitosan/gelatin composite</td>
<td><em>In vitro</em> culturing</td>
<td>Improved performance for 2 months</td>
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<td>Human HepG2 cell</td>
<td>Chitosan</td>
<td><em>In vitro</em> culturing on chitosan cast films</td>
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<td>Rat hepatocyte</td>
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<td>Rat hepatocyte</td>
<td>Chitosan nanofiber scaffolds</td>
<td><em>In vitro</em> culture in 3D chitosan-based nanofiber scaffolds</td>
<td>Significantly enhanced cell attachment and spreading, could maintain liver-specific functions for prolonged periods of time</td>
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injury alleviation, after which the cells exhibited a gene expression profile signature similar to that of adult human livers (Fig. 2).

Decellularized matrix

A decellularized matrix scaffold, which is procured by a decellularization technique that removes parenchymal cells from tissues or organs, can potentially retain the skeletal architecture and functional characteristics of the original tissue. Decellularized scaffolds derived from animal organs are now being explored as a promising resource for generating transplantable and functional organs. Lin et al. documented that a rat primary hepatocyte culture in a porcine liver-derived biomatrix for up to 45 days showed better liver-specific functions, such as albumin synthesis, urea production, and cytochrome P450 1A1 activity, than those on adsorbed collagen cultures. Uygun et al. seeded hepatocytes in a decellularized whole liver tissue. In vitro, the recellularized graft supported liver-specific functions including albumin secretion, urea synthesis and cytochrome P450 expression at levels comparable to a normal liver. When transplanted into rats, the recellularized liver grafts could support hepatocyte survival and function with minimal ischemic damage. Park et al. reported that they reseeded

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<tr>
<td>Rat hepatocyte</td>
<td>Chitosan/galactosylated hyaluronic acid/heparin scaffolds</td>
<td>In vitro culturing</td>
<td>Significantly improved the microenvironment of cell growth and prolonged liver functions such as albumin secretion, urea synthesis and ammonia elimination</td>
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<td>Human Huh-7 cell</td>
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<td>Murine iPSCs and ESCs</td>
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<td>Encapsulated into a 3D alginate MCG system</td>
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<td>Acute liver failure rat</td>
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<td>UCSCs-derived HLCs encapsulated in high mannuronic alginate scaffolds for the treatment of CCl4-induced ALF</td>
<td>Effectively attenuated biochemical tests, improved liver cytoarchitecture, increased expression of ALB and reduced AFP expression</td>
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Synthetic polymers

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<td>Fetal human liver cell</td>
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<td>Fetal rat liver cell</td>
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BMSCs, bone marrow mesenchymal stem cells; MSC, mesenchymal stem cells; DSM, decellularized spleen matrix; HUVECs, human umbilical vein endothelial cells; iPSCs, induced pluripotent stem cells; ADSCs, adipose-derived stem cells; BMSCs, bone marrow-derived stem cells; ESCs, embryonic stem cells; MCG, micro-cavitary hydrogel; UCSCs, umbilical cord stem cells; HLCs, hepatocyte-like cells; ALF, acute liver failure; ALB, albumin; AFP, α-fetoprotein; PLLA, poly-L-lactic acid; PCL, poly(ε-caprolactone); PLGA, poly(lactic-co-glycolic acid).
hepatocytes derived from iPSCs that had been generated by porcine ear fibroblasts into decellularized liver scaffolds, using a continuous perfusion system to culture the recellularized liver. When the grafts were transplanted into rats for the short-term, they found that the grafts expressed hepatocyte markers and did not rupture. Furthermore, researchers have found that hepatocyte-like cells derived from human amniotic epithelial, bone marrow, and adipose stromal cells display enhanced functionality when cultured on a decellularized liver substrate.

To overcome the limitations due to the shortage of donor organs, Xiang et al. utilized the spleen as the source of a decellularized scaffold for liver regeneration. The decellularized spleen scaffold could support the location and survival of BMSCs within the 3D matrix. Moreover, the subcutaneous implantation of the decellularized scaffold presented good histocompatibility. Another study also proved that a bioartificial liver constructed by a heparin-coated decellularized spleen scaffold could support the location and survival of BMSCs within the 3D matrix. Moreover, the subcutaneous implantation of the decellularized scaffold presented good histocompatibility.

However, the strategy based on a decellularized matrix suffers from limitations including a severe shortage of donor organs, high cost of treatment, and lifetime immune suppression.

Natural macromolecules

Natural macromolecules that are derived from ECM have been widely investigated as tissue engineering scaffolds for the culture of liver cells, including proteins such as collagen and gelatin as well as polysaccharides such as chitosan and alginate. These macromolecules have shown good biocompatibility both in vitro and in vivo.

Collagen, especially type I collagen, is the main structural protein in the extracellular space in various connective tissues in animal bodies. The collagen matrix serves to mediate cell growth, differentiation, and survival, and tissue organization. A collagen gel could provide a 3D microenvironment for hepatocytes to sustain high liver-specific functions. Khodabandeh et al. cultured human Wharton’s jelly MSCs in 2D collagen films and 3D collagen scaffolds for 21 days. The cells cultured in the 3D collagen scaffolds showed a significant increase in claudin expression compared to a conventional monolayer culture and the 2D collagen scaffold. Their subsequent study showed that the expression of the hepatocyte markers were increased significantly when administering the liver extract from a 3D matrigel/collagen culture of MSCs. Nakai et al. tested the effect of culturing human iPSCs-derived endoderm cells on a collagen vitrigel membrane; they found collagen vitrigel had promoted the differentiation of cells into functional hepatocyte-like cells, as shown by the decrease of immature marker alpha-fetoprotein (AFP) levels and the concomitant increase in expression of mature hepatocyte transcription factors (such as ALB and ASGR1) and markers involved in liver function.
functions (such as transporters, cytochrome P450 enzymes, and phase II metabolic enzymes). Moreover, collagen gel could also be used in a hepatocyte entrapment culture as an effective model to interpret drug hepatotoxicity and drug metabolism in vivo.74

Gelatin is the partially hydrolyzed form of collagen with a chemical composition that is, in many respects, very similar to that of its parent collagen. Recently, gelatin-based scaffolds have been documented to increase cell attachment and differentiation. Vasanthan et al.75 developed galactose-containing scaffolds for hepatocyte culture, using a two-step procedure involving galactosylation and electrospinning. Such galactose-containing hydrogels promoted cell-cell and cell-hydrogel interactions, aiding cellular aggregation and leading to the formation of spheroids.75 Another research group utilized a gelatin cryogel scaffold in which the cells generated on this scaffold were found to be similar to standard hepatocyte-like cells with a higher expression of hepatocyte-specific markers and appropriate functional characteristics, such as increased levels of urea biosynthesis and glycogen storage.76 On this basis, the researchers optimized the aforementioned scaffold and improved it through laminin incorporation; they found that the cells generated on this scaffold were strikingly similar to a standard hepatocyte cell line in terms of the expression of hepatocyte markers.77 Moreover, gelatin was also employed to fabricate microcarriers with chitosan for hepatocyte culture,78 and improved the performance of hepatocytes for as long as 2 months.79

Chitosan is a linear polysaccharide which has good biocompatibility and wide applications in biomedical fields. In the study of Verma et al.,70 HepG2 cells cultured on chitosan cast films formed 3D spheroids and exhibited higher amounts of albumin and urea synthesis compared to a monolayer culture. Rat hepatocytes seeded on an electrospun scaffold of galactosylated chitosan formed immobile, 3D, flat aggregates, and exhibited superior cell bioactivity with high levels of liver-specific albumin secretion, urea synthesis, and cytochrome P450 enzyme expression.81 Rajendran et al.82 established a chitosan nanofiber scaffold via an electrospinning technique combined with a coculture system that consisted of hepatocytes and fibroblasts, and thus developed a new 3D liver model for the maintenance of long-term liver functions. To obtain better biocompatibility, chitosan can be further modified with other macromolecules, such as collagen,83 gelatin,78 alginate,84 and heparin.85 For example, Fan et al.86 proved that chitosan/galactosylated hyaluronic acid scaffolds were suitable for improving hepatocytes adhesion and maintaining liver function in a previous work. To adjust the interaction of hepatocytes with the scaffold, they further modified the materials by the incorporation of heparin, which induced the formation of cellular aggregates with enhanced liver-specific metabolic activities and increased cell density.85

Alginate is a widely employed biomaterial in different areas of drug delivery and tissue engineering because of its good biocompatibility and optimal chemical properties. Its gelation with calcium ions provides calcium-alginate scaffolds with mechanical stability and relative permeability. Hepatocytes could organize in aggregates within calcium-alginate scaffolds, which established real 3D hepatocyte architecture with cell polarity, cell junctions, and microvillus-lined channels with abundant bile canaliculi.87 Lin et al.88 demonstrated that bone marrow-derived MSCs cultured in alginate scaffolds for several days displayed several liver-specific markers, such as the expression of genes encoding alpha-fetoprotein, ALB, connexin 32, CYP7A1, and were capable of glycogen storage. To fabricate implantable constructs for liver regeneration, Lau et al.89 encapsulated murine embryoid bodies (EBs) into a 3D alginate microvascular hydrogel system for both EB formation and subsequent hepatic lineage differentiation. Urea and albumin production were found to be significantly higher compared to a monolayer culture, demonstrating the beneficial effects of the 3D engineered environment.89 Shteyer et al.90 reported alginate scaffolds significantly increased animal survival, prolonged alanine aminotransferase and aspartate transaminase serum levels, and were accompanied by improved liver histology after an extended partial hepectomy. Another study on acute liver failure in rats showed that umbilical cord stem cell-derived hepatocyte-like cells encapsulated in alginate scaffolds effectively attenuated biochemical tests, improved liver cytoarchitecture, increased the expression of albumin, and reduced the level of AFP.91 Additionally, a new study from Shao et al.92 constructed carbon nanotube nanocomposites via the layer-by-layer assembly of negatively charged multiwalled carbon nanotubes and positively charged poly (dimethyldiallylammonium chloride), and proved that they could provide a potent regulatory signal over neural stem cells.

However, natural macromolecules suffer from a limited range of properties due to difficult processing of bulk material and the possibility of inducing potentially dangerous immune responses when used as xenografts or allografts.93 Moreover, the extraction and purification of natural macromolecules also leads to significant batch-to-batch variations,94 which can lead to unpredictable cell behavior and consequently impact the performance of cell-based tissue-engineered devices.
SYNTHETIC POLYMERS

Biodegradable synthetic polymers have been attractive candidates for hepatocyte culture in vitro and for making implantable scaffolds in vivo. A major advantage of synthetic polymers over natural macromolecules is the easy control of their physiochemical and biological modifications. Some of the widely used synthetic polymers are polylactic acid, poly(lactide-co-glycolide) (PLGA), and poly(ethyleneglycol) (PEG).

The study of Hanada et al. indicated that a 3D culture using poly-L-lactic acid (PLLA) scaffolds with OSM remarkably enhanced the albumin production and cytochrome P450 1A1/2 capacity during the culture time. This observation was in agreement with those of Jiang et al. and Wang et al., where fetal mouse liver cells were cultured on 3D PLLA scaffolds in the presence of nicotinamide, dimethyl sulfoxide, and OSM. In addition, the in vivo implantation of such engineered liver tissue showed a remarkably higher presence of albumin-positive engrafted cells 15 days after the operation when compared to freshly isolated and cultured cells for 1 day. However, researchers are less likely to use pure PLLA because of poor biocompatibility, and because scaffolds fabricated from single polymers display poor mechanical properties and are not easy to handle. To overcome these limitations, many reports have indicated the possible application of PLLA/ poly(ε-caprolactone) blends.

Ranucci and Moghe cultured hepatocytes on porous foams of amorphous PLGA with a wide range of controlled pore-size distributions (approximately 1 to 100 microns) and found that foams with supercellular size voids (~67 microns) promoted the kinetics of 3D aggregation with the most rapid and sustained albumin secretory kinetics. Kasuya et al. developed a 3D stacked culture method using biodegradable PLGA membranes. The hepatocyte layers on the PLGA membranes would reorganize into a 3D stacked structure after PLGA degradation. In the study by Liu et al., ESCs were mixed with Matrigel and immediately seeded in a PLGA scaffold with the appropriate inducing factors. The system enabled the spontaneous formation of spheroids, which differentiated into hepatocyte-like cells as evidenced by the expression of liver-specific markers and proteins.

However, synthetic biodegradable polymers have poor characteristics for cell culture due to their hydrophobic characteristics and lack of bioactive cues. Therefore, their bioactivities should be improved by chemically grafting bioactive cues and/or modifying the surface with natural macromolecules.

Figure 3. Assembly of transplantable 3D liver tissue. Natural macromolecules and synthetic polymers can be used to construct a decellularized matrix scaffold. Differentiated liver cells (hepatocytes, Kupffer cells, hepatic stellate cells, liver sinusoidal endothelial cells, and cholangiocytes) are then mixed and transplanted into the matrix scaffold to allow organogenesis for possible transplantation. PLA, polylactic acid; PLGA, poly(lactide-co-glycolide); PEG, poly(ethyleneglycol).

developed avidin-biotin binding systems (ABBS) for the initial attachment of biotinylated HepG2 cells to avidin-adsorbed PLLA surfaces. ABBS assisted HepG2 cells to adhere to a PLLA surface, and the proliferation of these attached cells was comparable with those cultured on collagen. Moreover, the hepatic functions of the attached cells were not significantly changed.\textsuperscript{106} Brown et al.\textsuperscript{107} modified a nanofibrous PLGA electrospun scaffold with a type I collagen coating. The modified scaffold led to 10-fold greater albumin secretion, 4-fold higher urea synthesis, and elevated transcription of hepatocyte-specific CYP450 genes in primary human hepatocytes compared to the unmodified PLGA scaffolds.\textsuperscript{107} Similarly, Bierwolf et al.\textsuperscript{108} developed a collagen-coated PLLA electrospun nanofibrous scaffold which provide a good \textit{in vitro} microenvironment for new tissue regeneration of primary rat hepatocytes. Application of novel materials for liver matrix scaffold construction and organogenesis were illustrated in Figure 3.

CONCLUDING REMARKS

Establishing a transplantable artificial 3D liver is complicated. Numerous studies have proved that a single hepatocyte component may not be able to fulfill the physiologically required liver functions. Thus, a combination of hepatocytes, HSCs, BDECs, and LSECs is proposed to form vascularized liver tissues providing blood circulation and nutrient supply. Existing researches have shown several ways to force stem cells to differentiate into those kinds of cells, with the proper physical supports from novel biomaterials. Indeed, there are a number of problems that need to be overcome, such as 1) ensuring that the differentiation process occurs properly and completely, 2) constructing 3D biomaterials with proper hepatic structures and with controllable release of growth factors for the self-formation of the liver tissue, and 3) avoiding possible tumorigenicity. Addressing these concerns will definitely promote the basic and clinical development of novel strategies for treating various liver diseases.

Authors' contribution

All of the authors were responsible for the interpretation of data and drafting and critically revising the manuscript for important intellectual content.

Acknowledgements

This work was supported by the Major Science and Technology Projects of Guangdong Province (No. 2015B020225005) and the National Natural Science Foundation of China (No. 81970515, 81873573, 81800525).

REFERENCES

43. Rubio-Tomás T, Aguilar-Bravo B, Sancho-Bru P. Genetic lineage...
72. Khodabandeh Z, Vojdani Z, Talaei-Khozani T, Bahnarpoor S. Hepatogenic differentiation capacity of human Wharton’s jelly mesen-


78. German CL, Madhally SV. Type of endothelial cells affects HepaRG cell acetaminophen metabolism in both 2D and 3D porous scaffold cultures. J Appl Toxicol 2019;39:461-472.


Should physicians go out of the way to differentiate between acute hepatitis B and acute exacerbation of chronic hepatitis B?

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Keywords: Hepatitis B; Chronic hepatitis; Antibodies

The article by Lall et al. published in *Clinical and Molecular Hepatology* fuels the discussion on differentiating laboratory markers between acute hepatitis B (AHB) and acute exacerbation of chronic hepatitis B (CHB-AE). This small retrospective study, which included 172 patients with AHB (n=89) or CHB-AE (n=83), had numerous strengths such as relevantly good follow-up, baseline assessment of IgM anti-HBc level, prothrombin time (PT), HBV DNA level, as well as qHBsAg and HBeAg values. The median cut-off ratio of IgM anti-HBc was significantly higher in AHB (30.44) than in CHB-AE (8.63) (P<0.01). The mean PT international normalized ratio (INR) was significantly greater in CHB-AE (1.88±1.24) compared to AHB (1.62±0.17). However, such findings were not new, and they must be taken with caution for several reasons.

In clinical practice, the similarities between AHB and CHB-AE in both clinical and laboratory context make it difficult to distinguish between the two clinical entities. Nevertheless, differentiating between the two entities is important, as they have different prognoses and therapeutic strategies. Most patients with AHB recover spontaneously, and treatment may be required only in a small number of patients who progress to fulminant hepatitis. On the other hand, patients with CHB-AE generally need antiviral therapy, since hepatic decompensation may be developed in patients with cirrhosis, especially. According to this study, a value of 20.5 for signal cut-off of IgM anti-HBc and 1.27 INR can be used to differentiate between AHB and CHB-AE. The evaluation of IgM anti-HBc levels seems to be an interesting strategy for differentiating the two clinical entities. Likewise, some reports have proposed that IgM anti-HBc levels should be reconsidered to define AHB. However, the diversity of reference values suggested from various studies is remarkable. These findings make it difficult to establish a standard reference value as the cut-off level. Unfortunately, as the author mentioned, IgM anti-HBc test was not a quantitative assay but a semiquantitative assay in this study. Therefore, the lack of prospective study about the standardization...
of quantitative assays and any valid clinical threshold would make the use of this marker quite unreliable.\(^8\) Moreover, the presence of IgM anti-HBc, which is associated with AHB, is necessary but not sufficient to diagnose AHB. IgM anti-HBc can be detected during episodes of CHB-AE. It may be the result of inflammation and liver cell injury during flare-ups of hepatitis, with consequent release of high concentrations of the nucleocapsid protein. These proteins lead to the activation of pre-existing plasma cells which are released into the circulation, and eventually contribute to the secretion of IgM anti-HBc during acute exacerbation.\(^8\) It can also lead to a misdiagnosis of AHB.

In addition, PT is not effective in differentiating between two entities, due to its short half-life and data obtained only at a one-time point. In particular, the proportion of cirrhosis was more than 70% in CHB-AE. PT is affected by the presence of cirrhosis. Therefore, PT seems to have poor sensitivity and specificity to discriminate between two entities.

Among other diagnosis tools, the avidity index of IgG anti-HBc is defined as the strength of IgG binding to antigenic epitopes of hepatitis B virus (HBV).\(^9\) This increases as IgG matures. Therefore, the low avidity index of IgG anti-HBc is an indicator of AHB, and high avidity index refers to CHB-AE. Terkmani et al.\(^10\) reported that an avidity index ≤3.4 was highly predictive of AHB. A quantitative and kinetics analysis of HBsAg titer is also worth applying. In AHB, HBsAg titer disappears much faster.\(^12\) Various possible serological studies have been performed. A combination study of HBV DNA, HBsAg, and IgM anti-HBc quantification sought to find the best strategy to discriminate between AHB and CHB-AE. Most of the results showed that AHB tends to show high IgM anti-HBc and low serum HBV DNA and HBsAg titer compared to CHB-AE.\(^15\) These findings originate from a vigorous immune response in acute viral infection.

So, do we need to go out of the way to differentiate between AHB and CHB-AE in the era of high potent antiviral agents? The KASL clinical practice guidelines for the management of CHB proposed that nucleoside analogues can be initiated in patients with severe AHB (e.g., coagulopathy, severe jaundice, liver failure).\(^6\) Other patients with AHB can be free of the virus without antiviral therapy, and do not progress to chronic illness. In addition, Brahmania et al.\(^10\) reported that alanine aminotransferase (ALT) flares rarely lead to significant decompensation in CHB patients with minimal fibrosis. In this case, it might be prudent to monitor the patients, rather than treating them.

Likewise, in patients with HBeAg-positive or HBeAg-negative CHB, prompt antiviral therapy should be initiated in the case of acute exacerbation, with the elevation of ALT ≥5–10 times the upper limit of normal, and signs of liver failure such as jaundice, PT prolongation, ascites, or hepatic encephalopathy.\(^6\) Therefore, the indication of antiviral therapy is similar between the two entities. There is no urgent need to establish the criteria and additional strategies for correct classification of AHB or CHB-AE in the era of highly potent and safe antiviral agents.

However, although the amount of clinical interest is low, it would help the physician to understand the natural course of HBV to find the gold standard for better defining and differentiating AHB from CHB-AE. Hopefully, the combination of new biomarkers, such as hepatitis B core-related antigen, HBV RNA, and immunologic markers (cytokine, chemokine profiles), will lead to differential diagnosis between AHB and CHB-AE.

**Conflicts of Interest**

The author has no conflicts of interests to disclose.

**REFERENCES**

8. Huang YW, Lin CL, Chen PI, Lai MY, Kao JH, Chen DS. Higher cut-off


Should you advocate for hepatocellular carcinoma surveillance in patients with alcohol-related liver disease or non-alcoholic fatty liver disease?

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Keywords: Hepatocellular carcinoma; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Alcoholic liver disease; Surveillance

Although hepatocellular carcinoma (HCC) is mainly caused by hepatitis B or C virus infection, the incidence of HCC related to alcohol abuse or non-alcoholic fatty liver disease (NAFLD) has risen markedly in recent decades. The Global Burden of Disease Study 2015 reported that 30% of new primary liver cancer cases were attributable to alcohol.1 In Korea, 2.4–10.9% of HCC cases were attributed to alcohol use.2 In addition, a year-over-year increase in the proportion of HCC cases attributable to NAFLD has been documented in several epidemiological studies.3,4 It is likely that the relative contribution of alcohol or NAFLD to primary liver cancer will increase worldwide, due to the improved efficacy of anti-hepatitis B and C treatments.

Regarding this issue, Kumar et al.5 compared the demographic and tumor characteristics, receipt of surveillance program, and survival outcomes between patients with HCC related to non-alcoholic steatohepatitis (NASH) and alcoholic liver disease (ALD) who were selected from the prospective HCC database of Singapore General Hospital. Patients with NASH-related HCC were older, and had higher prevalence of features suggestive of metabolic syndromes such as diabetes and hyperlipidemia. Notably, the majority of cases caused by NASH or ALD were associated with deficient surveillance as well as HCC detected late after the development of symptoms. These delays resulted in advanced-stage tumors at diagnosis, which were not amenable to potentially curative therapy and predicted poor outcomes.

Although this study offers additional insight into the characteristics of HCC in patients with NASH or ALD, it still had some bias and limitations. First, there was no data to compare NASH or ALD-related HCC and those caused by more common etiologies, including hepatitis B and hepatitis C. Second, the criteria for NASH diagnosis used in this study were somewhat ambiguous. NASH is standardly defined as the presence of 5% hepatic steatosis and inflammation with hepatocyte injury (e.g., ballooning), with or without fibrosis.6 Although liver biopsy is an essential element in the diagnosis of NASH,7,8 this study did not mention any

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Abbreviations:
ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis

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Editor: Seung Up Kim, Yonsei University College of Medicine, Korea

Received: Feb. 28, 2020 / Accepted: Mar. 3, 2020

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histological examination of surrounding liver tissues. These concerns should be considered when interpreting the results of this study.

Currently, limited data are available to suggest an optimal guidance for surveilling patients with NAFLD/NASH or ALD for HCC. Indeed, less than one-fifth of the 99 patients included in this study were surveilled or screened for HCC. A recently updated American Gastroenterology Association clinical practice guideline recommends that HCC screening should be considered in all NAFLD patients with cirrhosis as well as advanced liver fibrosis, where non-invasive approaches combining serological tests with elastography examinations in staging liver fibrosis in NAFLD are preferred. On the other hand, a European multicenter study showed that 1- and 2-year cumulative incidence of HCC development in patients with alcoholic cirrhosis were up to 1.8% and 5.2%, respectively, which were high enough to justify surveillance for the disease in these patients. On the contrary, a need for regular surveillance remains questionable in non-cirrhotic patients with NASH or ALD. In this study, more than 10% of HCC patients had no feature of cirrhosis, and several previous studies also found that HCC may develop in NAFLD/NASH patients without cirrhotic background. However, the implementation of HCC surveillance is not formally accepted in non-cirrhotic patients with chronic liver disorders of any etiology, due to its low cost-effectiveness.

Future studies should address the factors on which to base an optimal frequency and intensity of surveillance for patients with advanced fibrosis or cirrhosis attributed to hepatic fat or alcohol.

Conflicts of Interest
The author has no conflicts to disclose.

REFERENCES

7. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402.
Toll-like receptor 9, a possible blocker of non-alcoholic steatohepatitis?

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Keywords: Non-alcoholic fatty liver disease; Toll-like receptor 9; Immune system; Interferon-gamma; T-cell

Toll-like receptor 9 (TLR9) is a transmembrane protein which is expressed on the cell surface of immune system cells such as T cells, macrophages, natural killer cells, and other antigen-presenting cells. TLR9 binds the cytosine-phosphate-guanine dideoxynucleotide motif within bacterial and viral DNA, and triggers signaling cascades that lead to a pro-inflammatory cytokine response, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6). Plasma from mice and patients with non-alcoholic steatohepatitis (NASH) contained high levels of mitochondrial DNA (mtDNA) from microparticles of hepatocyte origin and activated TLR9. TLR9 ligands, such as mtDNA, may activate the innate immune system through TLR9, which leads to the development of steatohepatitis, fibrosis, and insulin resistance.

The prevalence of nonalcoholic fatty liver disease (NAFLD) increases worldwide, and it is emerging as a major cause of chronic liver disease. Among the various phenotypes of NAFLD, NASH is highly likely to progress to the development of end-stage liver disease and cardiometabolic disease, resulting in both liver-related and non-liver-related mortality.

In this issue of Clinical and Molecular Hepatology, Alegre et al. reported that TLR9 expression on liver and peripheral T cells was the lowest in patients with simple steatosis (SS) and was positively associated with anthropometric, biochemical, and histopathological features of NAFLD. The authors interpreted the mechanism of an overall downregulation of TLR9 on T cells from SS patients as a protective adaptation from hepatocellular injury, whereas a constant, unvarying expression in patients with NASH was considered as a failure of this regulatory mechanism.

At baseline, patients with SS showed a low frequency of circulating type 1 CD8+ cells. In vitro co-stimulation of T cells in these patients induced a limited number of IFN-γ-producing CD8+ T cells. However, NASH patients showed a higher frequency of IFN-γ-producing cells compared to SS patients and controls at baseline. These results confirmed the previous data concerning a synergism between T cell receptors and TLR9 during the induction of IFN-γ.

IFN-γ is produced predominantly by natural killer cells as part of...
the innate immune response, and by CD4\(^+\) T helper cells and CD8\(^+\) cytotoxic T cells once antigen-specific immunity develops. It is an important activator of M1 macrophages (classically activated macrophages), which play an important role as the pro-inflammatory type in phagocytosis and secretion of pro-inflammatory cytokines. Inflammation driven by M1 macrophages is counterbalanced by alternatively polarized M2 macrophages that promote the resolution of inflammation and tissue repair.\(^{10,11}\) Wan et al.\(^{12}\) suggested that promoting M2-induced M1 Kupffer cell apoptosis might prove to be a relevant strategy to limit high fat-induced inflammation and hepatocyte injury.

Innate immune signaling has been considered to play a key role in initiating and developing hepatic inflammation, contributing to the transition from nonalcoholic fatty liver to NASH. Recently, Garcia-Martinez et al.\(^{4}\) tried TLR7/9 antagonist IRS954 to block the ability of hepatocyte mtDNA from high fat diet fed mice to activate proinflammatory cytokines in primary macrophages. IRS954 led to a significant reduction in histological NAFLD parameters, including steatosis, ballooning, and inflammation, and also reduced pro-IL-1\(\beta\), IL-6, TNF-\(\alpha\), and serum alanine aminotransferase levels.

In summary, intrahepatic and peripheral TLR9 protein expressions were the lowest in patients with SS, which was likely a protective adaptation from hepatocellular injury. On the other hand, the constant expression in patients with NASH may be a failure of this regulatory mechanism. However, NASH patients showed a higher frequency of IFN-\(\gamma\) producing cells compared to SS patients and controls at baseline. TLR9 activation initiates the innate immune system, which leads to the development of steatohepatitis, fibrosis, and insulin resistance.

Blocking TLR9 may reverse NASH and be a possible therapeutic target for the blocking of NASH. Further study is needed to examine the effect of TLR9 ablation and TLR9 antagonism on T cells, as well as to investigate the role of TLR9 in the activation of stellate cells and fibrosis.

Acknowledgements
This study was supported by the Soonchunhyang University Research Fund.

Conflicts of Interest
The author has no conflicts to disclose.

REFERENCES
The dilemma of differentiating between acute hepatitis B and chronic hepatitis B with acute exacerbation: Is quantitative serology the answer?

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Abbreviations:
- AHB, acute hepatitis B
- ALT, alanine aminotransferase
- anti-HBe, antibody to HBeAg
- APASL, Asian Pacific Association for the Study of the Liver
- APRI, AST platelet ratio index
- AST, aspartate aminotransferase
- AUROC, areas under ROC
- CHB-AE, acute exacerbations in chronic hepatitis B
- CLIA, Chemiluminescence Immunoassay
- HBeAg, hepatitis B e antigen
- HBsAg, hepatitis B surface antigen
- HBV, hepatitis B virus
- IgM anti-HBc, immunoglobulin M antibody to the hepatitis B core antigen
- INR, international normalized ratio
- NPV, negative predictive value
- PEI, Paul-Ehrlich Units
- PPV, positive predictive value
- qHBsAg, quantitative HBsAg
- RLU, relative light units
- ROC, receiver operating characteristic
- S/Co, signal cut-off
- SPSS, Statistical Package for the Social Sciences

Graphical Abstract

A value of cutoff at 20.6 S/Co of IgM anti HBc and 1.27 INR can differentiate between AHB and CHB-AE.

Editor: Hyun Woong Lee, Yonsei University College of Medicine, Korea

Received: Jun. 25, 2019 / Revised: Oct. 14, 2019 / Accepted: Dec. 2, 2019

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**INTRODUCTION**

Hepatitis B virus (HBV) infection is a substantial global health problem, with more than 2 billion people infected worldwide and 257 million cases being chronic hepatitis.\(^1,2\) In India, it is estimated that about 40 million people are chronically infected with the virus, with a prevalence of 3.7%.\(^3\) HBV infection results in a spectrum of disease entities ranging from asymptomatic carrier state to the most severe form of chronic active hepatitis B.\(^4\)

A large percentage of patients with chronic active hepatitis may frequently show a changing pattern, including acute exacerbations in liver injury along with episodes of normal liver function. This reflects the dynamic interplay between the immune response of human body and viral replication.\(^7\) According to the 2015 Asian Pacific Association for the Study of the Liver (APASL) clinical practice guidelines on the management of hepatitis B, acute exacerbations in chronic hepatitis B (CHB-AEs) are usually defined as intermittent elevations of aminotransferase to more than five times the upper limit of normal and more than twice the baseline value.\(^6\) In HBV endemic areas, CHB-AEs are common and may often be the first sign of the disease. Over 50% of such patients are presumed to have acute hepatitis B (AHB) due to similar clinical and serological pictures, thereby posing a major diagnostic dilemma.\(^7,10\)

The need to differentiate between CHB-AE and AHB is worthy of our attention due to their different prognosis which requires different types of therapeutic intervention. Most of the patients with AHB show complete clinical improvement and resolve spontaneously. Only a small number of those with severe/fulminant disease may require treatment while those with CHB-AE usually require therapy for hepatic decompensation, and high mortality may occur as a result of hepatocellular dysfunction.\(^11\) Here, a simple and reliable serological marker would come in handy to differentiate between these two conditions. The amount of available data on the use of serological assays in diagnosing CHB-AE is very limited, and there are variations in the inclusion and differentiation criteria of different studies. Immunoglobulin M antibody to the hepatitis B core antigen (IgM anti-HBc) has been considered an important diagnostic marker for AHB.\(^12,13\) However, since about 20% to 27.5% of CHB-AE patients have IgM anti-HBc positivity with the fully automated, quantitative analysis method, these pa-
tients could clinically present as AHB. Therefore, a simple, effective, and reliable serological marker with a cut-off value is required to differentiate between these two conditions. Very few studies on this topic have been conducted in India. In this study, we aimed to identify the difference in serological markers of AHB and CHB-AE, and to figure out an optimal cut-off value for the serological monitoring of HBV.

**MATERIALS AND METHODS**

This study was performed at a tertiary care liver center in India. After the approval from the Institutional Ethics Committee (IEC/2018/58/MA03) and review board, a retrospective analysis of records was done on all patients who presented with acute viral hepatitis with hepatitis B surface antigen (HBsAg) and IgM anti-HBc positivity between May 2015 and May 2017. Detailed clinical information regarding the onset of illness, presenting signs and symptoms, and previous history of such episodes of HBV infection were obtained from the Hospital Information System. Patients co-infected with hepatitis A virus, hepatitis C virus, hepatitis E virus, and human immunodeficiency virus were excluded from the study. After careful review of the subjects’ history as well as their clinical and serological profiles, 172 patients were included and divided into two groups: AHB (89, 51.7%) and CHB-AE (83, 48.3%). AHB group included patients with clinical signs and symptoms of AHB without a history of past HBV infection, as well as HBsAg not persisting for more than 6 months. CHB-AE group included HBV carriers with HBsAg antigen persisting for more than 6 months, who had more than five times the upper limit of normal and more than twice the baseline value of aminotransferase. Baseline characteristics such as age, sex, and biochemical parameters including serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, international normalized ratio (INR), AST platelet ratio index (APRI) score, and albumin, were noted. Out of 83 patients from CHB-AE group, 60 (72.2%) were cirrhotic and 23 (27.7%) were non-cirrhotic using 1.5 as the higher cut-off of APRI score. Biochemical and virological profiles of the two groups were compared to establish the diagnostic markers for their differentiation.

Various serological markers, such as IgM anti-HBc, quantitative HBsAg (qHBsAg), hepatitis B e antigen (HBeAg), and antibody to HBeAg (anti-HBe), were tested on commercial Chemiluminescence Immunoassay (CLIA)-based platform (Architect i1000SR; Abbott Diagnostics, Abbott Park, IL, USA). Chemiluminescence immunoassay on the Abbott Architect is a two-step immunoassay with flexible assay protocols called chemiflex, which utilizes paramagnetic particles coated with the respective antigen/antibodies to which human serum is incubated. After washing, acridine-labelled conjugate was added followed by pre-trigger and trigger solutions, and the resulting chemiluminiscent reaction was measured as relative light units (RLUs). A direct relationship was shown between the amount of analyte in the sample and the RLUs detected by Architect System optics. Results were calculated as normalized

<table>
<thead>
<tr>
<th>Variable</th>
<th>AHB</th>
<th>CHB-AE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>66/23</td>
<td>65/18</td>
<td>0.52</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43±16</td>
<td>43±17</td>
<td>0.95</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL) (NR, 0.1–1.2)</td>
<td>17.7 (7.1–22.9)</td>
<td>15.4 (4.3–26.5)</td>
<td>0.996</td>
</tr>
<tr>
<td>AST (IU/L) (NR, 10–40)</td>
<td>529 (193–1,021)</td>
<td>221 (93–269)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L) (NR, 7–56)</td>
<td>664 (177–1,243)</td>
<td>168 (87–583)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>0.98±0.09</td>
<td>1.03±0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>INR</td>
<td>1.62±0.17</td>
<td>1.88±1.24</td>
<td>0.045</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.8±2.3</td>
<td>12.0±2.3</td>
<td>0.34</td>
</tr>
<tr>
<td>Platelet (×10^3/µL)</td>
<td>224 (151–272)</td>
<td>213 (111–291)</td>
<td>0.09</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.2±0.8</td>
<td>2.9±0.8</td>
<td>0.01</td>
</tr>
<tr>
<td>APRI</td>
<td>6.5 (2.9–11.6)</td>
<td>4.1 (1.6–9.2)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or median (IQ range).

AHB, acute hepatitis B; CHB-AE, chronic hepatitis B with acute exacerbation; NR, normal range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; Hb, hemoglobin; APRI, AST platelet ratio index; IQ, interquartile range from the 25th (Q1) to the 75th (Q3) percentile.
signal cut-off (S/Co) ratios obtained by measuring the signal strength of sample and the signal strength of an internal cut-off. IgM anti-HBc positivity was defined by an S/Co ratio $\geq 1.0$. The concentration of qHBsAg was determined using a previously generated Architect HbsAg calibration curve, which allows the quantitation of HbsAg from 0.05 to 250 IU/mL. Further dilution of samples was done to quantitate higher values. S/Co $\geq 1$ was considered reactive for HBeAg, while S/Co $\leq 1$ was considered as reactive for anti-HBe. Real time polymerase chain reaction was done for HBV DNA quantitation using COBAS TaqMan HBV test (Roche Diagnostics; GmbH, Mannheim, Germany), which has a lower limit of detection of 6 IU/mL and linear range of 29 to $1.1 \times 10^8$ IU/mL.

**Statistical analysis**

Quantitative variables were expressed as mean±standard deviation, or as median with range. Qualitative variables were expressed as numbers with percentage. HBV DNA levels and qHBsAg values were logarithmically transformed for analysis. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software for Windows version 22.0 (IBM SPSS, Armonk, NY, USA). Categorical data were compared using chi square test or Fischer exact test, as appropriate. Continuous data between the two groups were compared using Student’s t test or Mann-Whitney test, as appropriate. P value $<0.05$ was considered statistically significant. To identify the independent factors that were significantly associated with AHB, multiple logistic regression analysis was conducted using forward stepwise likelihood ratio. Selected variables with P value $<0.05$ on univariate analysis were included in regression analysis. To determine the optimal cut-off value of the variables for differentiating AHB from CHB-AE, the receiver operating characteristic (ROC) curves were plotted. The areas under ROC (AUROC) curves of identified factors were calculated. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated.

**RESULTS**

Baseline characteristics of the study population are described in Table 1. The enrolled population was divided into two groups: AHB (89, 51.7%) and CHB-AE (83, 48.3%). Both of the groups were comparable in all parameters except for AST and ALT being significantly higher in AHB group, reflecting the intensity of necroinflammation, and INR was significantly greater in CHB-AE group.

**Comparison of virological parameters**

Various viral markers were compared between the two groups (Table 2). To decrease the variability of data and make data conform more closely to the normal distribution, HBV DNA values were log-transformed as $\log_{10}$ IU/mL. No significant difference was found between AHB and CHB-AE patients in terms of median values of viral markers, based on semiquantitative analysis of test results. However, S/Co values between the two groups differed significantly in median levels of IgM anti-HBc (30.4 vs. 8.6, $P<0.001$).

**Finding independent predictors for CHB-AE in comparison to AHB**

Multiple logistic regression analysis of various factors revealed higher IgM anti-HBc titers and low INR as independent predictors of AHB as compared to CHB-AE (Table 3). Greater than 80% of correct predicting capability was seen in the combination of these two markers.

Table 2. Comparison of virological markers between groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>AHB (n=89)</th>
<th>CHB-AE (n=83)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg, S/Co</td>
<td>33.5 (8.5–115.0)</td>
<td>38.9 (2.0–1,062.2)</td>
<td>0.32</td>
</tr>
<tr>
<td>qHBsAg (log$_{10}$ IU/mL)</td>
<td>4.1 (3.3–4.7)</td>
<td>1.1 (3.3–4.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>HBV DNA viral load (log$_{10}$ IU/mL)</td>
<td>4.6 (3.4–5.6)</td>
<td>4.3 (5.8–3.2)</td>
<td>0.75</td>
</tr>
<tr>
<td>IgM anti-HBc, S/Co</td>
<td>30.4±8.5</td>
<td>8.6±11.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as median (IQ range) or mean±standard deviation. Values of qHBsAg and HBV DNA were log-transformed for analysis. AHB, acute hepatitis B; CHB-AE, chronic hepatitis B with acute exacerbation; HBeAg, hepatitis B e antigen; S/Co, signal cut-off; qHBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; IgM anti-HBc, immunoglobulin M antibody to hepatitis B core antigen; IQ range, interquartile range.
Diagnostic value of IgM anti-HBc and INR as independent predictors alone and in combination for the differentiation between AHB and CHB-AE

To determine the cut-off value of IgM anti-HBc as a sensitive marker of AHB, ROC was plotted. Figure 1 shows AUROC (0.87) using the sensitivity of IgM anti-HBc at various cut-off points. The sensitivity and specificity for the cut-off value of 20.5 were 93.3% and 92.7%, respectively, while PPV and NPV at this cut-off were 86.9% and 95.9%, respectively. The diagnostic performance of various S/Co ratios of IgM anti-HBc was evaluated based on the distribution of patients in each group (Table 4). IgM anti-HBc S/Co ratios of <10 and >30 were found to be significant markers for CHB-AE and AHB, respectively (P<0.001). To determine the cut-off value of INR as a sensitive marker of AHB, ROC was plotted. Figure 2 shows AUROC (0.56) using the sensitivity of INR at various cut-off points. The sensitivity and specificity for the cut-off value of 1.27 were 57.9% and 45.1%, respectively. After combining these two markers, AUROC slightly increased to 0.90, while sensitivity decreased to 80.2% and specificity remained almost the same (92.1%; Fig. 3). These results showed that combining INR to IgM anti-HBc did not improve its predicting capability as an independent marker.

Table 3. Multivariate logistic regression analysis for predicting AHB in comparison to CHB-AE (both host and viral factors)

<table>
<thead>
<tr>
<th>Significant variable</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM anti HBc, S/Co</td>
<td>&lt;0.001</td>
<td>0.9</td>
<td>0.8–0.9</td>
</tr>
<tr>
<td>INR</td>
<td>0.01</td>
<td>1.8</td>
<td>1.1–2.2</td>
</tr>
</tbody>
</table>

AHB, acute hepatitis B; CHB-AE, chronic hepatitis B with acute exacerbation; IgM anti-HBc, immunoglobulin M antibody to hepatitis B core antigen; S/Co, signal cut-off; INR, international normalized ratio.

Table 4. Distribution of subjects with different S/Co of IgM anti-HBc

<table>
<thead>
<tr>
<th>S/Co</th>
<th>AHB</th>
<th>CHB-AE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>2</td>
<td>49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>12</td>
<td>17</td>
<td>0.23</td>
</tr>
<tr>
<td>20–30</td>
<td>27</td>
<td>8</td>
<td>0.14</td>
</tr>
<tr>
<td>&gt;30</td>
<td>48</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>83</td>
<td>172</td>
</tr>
</tbody>
</table>

S/Co, signal cut-off; IgM anti-HBc, immunoglobulin M antibody to hepatitis B core antigen; AHB, acute hepatitis B; CHB-AE, chronic hepatitis B with acute exacerbation.

Figure 1. Receiver operating characteristic curve (ROC) plotted to determine the cut-off value of IgM anti-HBc as a sensitive marker of AHB. Figure shows the area under the ROC curve (AUROC) using the sensitivity of IgM anti-HBc at various cut-off points. IgM anti-HBc, immunoglobulin M antibody to hepatitis B core antigen; AHB, acute hepatitis B.

Figure 2. Receiver operating characteristic curve (ROC) plotted to determine the cut-off value of international normalized ratio (INR) as sensitive marker of acute hepatitis B. Figure shows the area under the ROC curve (AUROC) using the sensitivity of INR at various cut-off points.
Diagnostic value of HBV DNA levels for the differentiation between AHB and CHB-AE

Although the difference between the two groups for log10 HBV DNA values was not statistically significant, ROC was plotted to understand the role of HBV DNA levels as well as to find out the cut-off value of HBV DNA levels as a predictor of AHB. AUROC using log10 HBV DNA at various cut-off points was calculated. A cut-off value of $\leq 10.5 \log_{10}$ IU/mL could predict AHB with a sensitivity of 51.3%, specificity of 51.4%, and PPV of 50.7. HBV DNA viral load was not significantly different between the two groups.

Role of combining virological markers in the differentiation between AHB and CHB-AE

The diagnostic efficacy of serological markers, such as qHBsAg, HBeAg, and HBV DNA, in combination with IgM anti-HBc at their respective cut-off points was analyzed. After combining a cut-off of 10.5 log10 IU/mL for HBV DNA and 2.7 log10 IU/mL for qHBsAg, the sensitivity increased from 97.8% to 98.9% while the specificity significantly decreased from 51.8% to 49.1%, as compared to using IgM anti-HBc alone. Similarly, by combining HBeAg S/Co (cut-off value of 1.5), the specificity also decreased (13.8%). These results showed that combining virological markers did not prove to be a better indicator to differentiate AHB from CHB-AE, as compared to using IgM anti-HBc alone.

DISCUSSION

The present study was performed to understand the role of various virological markers in differentiating AHB from CHB-AE. Both AHB and CHB-AE resemble each other in clinical presentation as well as in terms of biochemical characteristics. For differentiating this scenario on initial presentation, appropriate clinical history of the patients is required to highlight the onset of symptoms, of which is often lacking and thus isn’t helpful in many cases. For numerous decades, the qualitative estimation of serological markers of hepatitis B infection has been the mainstay of diagnosis, in addition to the evaluation of AST and HBV DNA. Qualitative estimation using old enzyme immunoassays, which are usually standardized at a higher threshold values, lack the sensitivity to detect the variable at lower concentrations; furthermore, AST values lack specificity that represents the state of hepatic necrosis only. For many years, IgM anti-HBc was considered a specific marker for AHB due to the old assays that were standardized at 600–700 PEI (Paul-Ehrlich Units) or avidity index, which detected only high values. However, with the help of the latest diagnostic techniques like chemiluminescence, quantitation of this parameter on well-characterized specimens of acute and CHB revealed a considerable percentage of positive cases among chronic hepatitis cases, but with lower levels. This has been demonstrated in various studies, as shown in Table 5.

In our study, the median levels of IgM anti-HBc and INR were found to be significantly different between the two groups, and also proven to be a sensitive predictor for AHB group. The result of the present study is in accordance with previous studies, which used different methods of quantification for IgM anti-HBc against semiquantitative serology. Higher IgM anti-HBc titers suggest a highly active immune response which promotes B cell differentiation into IgM producing plasmablasts. Since our hospital is a tertiary care liver institute, the presentation of majority cases in early acute phase of the illness can be responsible for the higher cut-off value obtained in our study, or it can also be due to the inherent immunological difference by region. The serum level of INR, which reflect the degree of coagulopathy in a patient with hepatic disease, was an independent predictor for AHB, although it had a
lesser sensitivity compared to IgM anti-HBc. This result could not be inferred in isolation of other coagulation parameters such as platelets, prothrombin time, and activated partial thromboplastin time. We could not follow up with the patients for evaluating the dynamic values of INR using the prescribed drug regimen. Further studies are required to confirm our findings.

The present study did not find any significant differences between HBV DNA levels of both groups. This was in contrast to other studies which reported higher levels of HBV DNA being associated with CHB-AE compared to AHB. HBV DNA levels in CHB-AE group in our study were quite comparable to those of AHB group. This could be due to the non-immunosuppressed state of the subjects, rapid immune clearance following HBV reactivation, effect of antiviral treatment which suppresses viral replication, and misallocation of some subjects in the two groups, as they were not fully clinically assessed.

HBeAg positivity has been found more frequently in patients with acute infection than in those with chronic infection, but the difference is not statistically significant. The present study showed no significant difference between the values of HBeAg in the two groups. Recent data support the fact that high levels of HBsAg are associated with viral replication and disease activity. In acute hepatitis, the levels of HBsAg are generally above 1×10^7 IU/L, and they decrease in the recovery phase. Although a median value of qHBsAg (4.12 log_{10} IU/mL) was higher in AHB than in CHB-AE (1.09 log_{10} IU/mL) in the present study, the association was not statistically significant. Various studies have reflected significantly higher values of qHBsAg for acute phase.

The liver fibrosis status in the two groups was not compared using Fibroscan, FIB-4 index, and M2BPGi. However, the APRI score and AST/ALT ratio did not differ between the two groups. While AST/ALT ratio >1 is recommended as a predictor of cirrhosis and has sensitivity and specificity of 81.3% and 55.3%, respectively, an APRI of more than 1.5 has AUROC of 80% and 89%, respectively, for advanced fibrosis F3–F4 and cirrhosis. Numerous studies have highlighted that the data concerning the clinical utility of transient elastography in hepatitis B appears promising with 84% and 65% positive and NPVs, respectively, for a cut-off of 7.0 kPa. Although acute hepatitis can produce false-positive results in the APRI, Forns index, FIB-4, or Fibrometer tests, which all measure the levels of aminotransferases, not much is present in the existing literature on differentiating AHB from CHB-AE using

### Table 5. Comparative analysis of various studies evaluating the role of serology for differentiating between AHB and CHB-AE

<table>
<thead>
<tr>
<th>Study</th>
<th>Year/location</th>
<th>Technique</th>
<th>IgM anti HBc</th>
<th>HBV DNA</th>
<th>Other relevant findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodella et al.</td>
<td>2006/Italy</td>
<td>Chemiluminescent immunoassay</td>
<td>S/Co=10, avidity index=0.7 diagnostic for AHB</td>
<td>Not done</td>
<td>HBsAg levels differed significantly AHB &gt; CHB-AE</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2006/Taiwan</td>
<td>Serology: MEIA HBV DNA-qPCR</td>
<td>Mean index value 2.9 AHB vs. 1.5 CHB-AE</td>
<td>Comparable in both groups</td>
<td>Cut-off for IgM anti-HBc 2.4–2.5 showed sensitivity and specificity 90%</td>
</tr>
<tr>
<td>Han et al.</td>
<td>2008/Shanghai</td>
<td>Serology: enzyme immunoassay HBV DNA-qPCR</td>
<td>At 1:10,000 titer high sensitivity and specificity of 96.2% and 93%</td>
<td>No significant difference</td>
<td>Combining HBV DNA + HBeAg increases diagnostic power Low HBeAg level more useful than negative HBeAg</td>
</tr>
<tr>
<td>Kumar et al.</td>
<td>2006/India</td>
<td>Enzyme immunoassay</td>
<td>Titer &gt;1,000 seen in 80% people of AHB</td>
<td>&lt;0.5 pg/mL Seen in CHB-AE</td>
<td></td>
</tr>
<tr>
<td>Dao et al.</td>
<td>2012</td>
<td>Enzyme immunoassay</td>
<td>S/N higher in AHB=88.2</td>
<td>3.9 log_{10} IU/mL vs. 5.2 log_{10} IU/mL for AHB vs. CHB-AE</td>
<td>Cut point S/N ratio of 5.0 for IgM</td>
</tr>
<tr>
<td>Park et al.</td>
<td>2015</td>
<td>Chemiluminescent immunoassay</td>
<td>S/Co ratio of IgM Anti-HBc was significantly higher in AHB group</td>
<td>HBV DNA level was significantly higher in CHB-AE group</td>
<td>The optimal cut-off values of IgM anti-HBc and HBV DNA levels for differentiating the two conditions were 8 S/Co ratio and 5.5 log_{10} IU/mL, respectively</td>
</tr>
</tbody>
</table>

AHB, acute hepatitis B; CHB-AE, chronic hepatitis B with acute exacerbation; IgM anti-HBc, immunoglobulin M antibody to hepatitis B core antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; S/Co, signal cut-off; qPCR, quantitative real-time polymerase chain reaction; MEIA, microparticle enzyme immunoassay; S/N, signal by noise ratio; qHBsAg, quantitative hepatitis B surface antigen.
these markers. Although a few studies have highlighted the importance of combining IgM anti-HBc and HBV DNA to increase the sensitivity and specificity of newly created marker, the present study could not find such association. Although an increase in sensitivity was seen after combining IgM anti-HBc with qHBsAg, HBV DNA, and HBeAg, the specificity decreased remarkably. This could be due to the fact that not all of the subjects in the study underwent testing for all serological markers during follow-up visits to the hospital.

In conclusion, CHB-AE causing derangement of liver functions may be seen in a flare of HBV during the immune clearance phase or HBV reactivation in patients with inactive hepatitis infection or resolved HBV infection. In endemic countries like India, the differentiation between CHB-AE and AHB is important for both prognostication and management of the disease. Quantitation of IgM anti-HBc can work as a simple marker of differentiation between AHB and CHB-AE. Our analysis of various serological markers in this study showed that only IgM anti-HBc was a significant discriminating factor between CHB-AE and AHB, and that combining other markers did not add to its discriminating power.

**Study limitations**

The present study had some limitations due to its retrospective nature, and it was purely dependent on the patient information that were available in the hospital system. To find the best possible operational and feasible approach, the patients were retrospectively categorized into two groups based on their clinical history as well as the presence or absence of HBsAg antigen after 6 months.

Also, the possibility of misclassification of cases cannot be negated, as the loss of HBsAg at exactly 6 months is not very commonly reported. Therefore, further studies are needed to highlight the role of serological markers in differentiating AHB and CHB-AE. Furthermore, the evaluation of cut-off of IgM anti-HBc by quantitative assay is recommended over the semiquantitative one used in this study for better statistical agreement, and INR results on serial follow-up would add more to our knowledge.

**Authors’ contribution**

Ekta Gupta: Conception, designing, and supervision of work.
Writing and revision of manuscript

Sujata Lall, Pragya Agarwala: Designing, writing, and revision of manuscript

Guresh Kumar: Statistical analysis and interpretation of data

Manoj Kumar Sharma: Critical revision of the manuscript

**Conflicts of Interest**

The authors have no conflicts of interest to disclose.

**REFERENCES**


8. Chu CM, Sheen IS, Liaw YF. The aetiology of acute hepatitis in Taiwan: acute hepatitis superimposed on HBsAg carrier state as the main aetiology of acute hepatitis in areas with high HBsAg carrier rate. Infection 1988;16:233-237.


14. Santos MV, Duarte MIS, Barone AA. Acute exacerbation in chronic
Comparisons between non-alcoholic steatohepatitis and alcohol-related hepatocellular carcinoma

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Graphical Abstract

Non-alcoholic and alcoholic steatohepatitis both cause cirrhosis and liver cancer but differences between NASH-HCC and ASH-HCC are unknown.

NASH-HCC patients were older at diagnosis, had significantly more metabolic risk factors and were less frequently diagnosed on surveillance compared to ASH-HCC.

18.5% of NASH and 11.1% of ASH patients were non-cirrhotic at HCC diagnosis.

Most NASH-HCC patients were diagnosed at the symptomatic stage.

There should be better HCC surveillance than current practice in NASH patients.

Abbreviations:
AFP, alpha-fetoprotein; Alb, albumin; ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, aspartate transaminase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; BMI, body mass index; CT, computerized tomography; CTP, Child-Turcotte-Pugh; DM, diabetes mellitus; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MELD, model for end-stage liver disease; MRI, magnetic resonance imaging; NASH, non-alcoholic fatty liver disease; NASH-HCC, non-alcoholic steatohepatitis HCC; OS, overall survival; PTEN, phosphatase and tensin homolog; Tbil, total bilirubin; TNM, Tumor lymph Node Metastasis

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Editor: Ju Hyun Shim, University of Ulsan College of Medicine, Korea

Received: Jan. 24, 2019 / Revised: Jul. 4, 2019 / Accepted: Jul. 29, 2019

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INTRODUCTION

Globally, liver cancer is the 5th and 9th most common malignancy in men and women respectively, representing the 2nd leading cause of cancer mortality. Significant burden of hepatocellular carcinoma (HCC) is seen in Asia.\(^1\,^2\) While viral hepatitis traditionally accounts for most cases of HCC, a burgeoning proportion of HCC is associated with non-alcoholic fatty liver disease (NAFLD) or what was labelled cryptogenic previously.\(^3\) It is increasingly recognised that cryptogenic cirrhosis often represents “burnt-out” NAFLD as a significant number of these patients would have the clinical phenotype comparable with NAFLD, such as higher prevalence of metabolic risk factors, but not the characteristic histopathologic features of NAFLD, which typically disappear with the onset of advanced fibrosis/cirrhosis.\(^4\) NAFLD is a lifestyle-related condition affecting the liver and is considered as the hepatic manifestation of metabolic syndrome due to its association with diabetes mellitus (DM), dyslipidemia, insulin resistance and obesity. NAFLD embodies a spectrum of disease ranging from simple steatosis, considered benign accumulation of lipids within the liver to the more aggressive non-alcoholic steatohepatitis (NASH), characterized by necrosis, inflammation, and fibrosis, with the potential to progress to liver cirrhosis and HCC.\(^5\,^6\) NAFLD is regarded as the most common cause of chronic liver disease in the United States and other developed countries.\(^7\) 

While the natural history of NAFLD/NASH is still not fully understood, it shares certain similarities with alcoholic liver disease (ALD) and ASH.\(^8\) In patients with NASH, HCC usually occurs in the setting of liver cirrhosis. However, it is recognized that HCC can develop in non-cirrhotic NASH as well. Over a follow-up period of 7–12 years, the rates of liver-related complications for NAFLD are 3–5% for cirrhosis, 2% for jaundice and encephalopathy, 1% for...
variceal bleeding, and 0.5–1% for development of HCC. A multicenter longitudinal cohort study of biopsy-proven NAFLD reported the outcome of 619 patients over a median follow-up period of 12.6 years - 44 patients (7%) developed liver-related events including five patients (0.8%) diagnosed with HCC. Of the 44 patients who developed the end-stage liver disease, 17 (38.6%) died from complications of liver disease.

Similarly, ALD is an established risk factor for the development of cirrhosis and HCC. The lifetime risk of development of liver cirrhosis is 8–20% and that of HCC 3–10% with ongoing alcohol abuse. Chronic alcohol use of more than 80 g/day for more than 10 years increases the risk of HCC by 5-fold. In a meta-analysis, there was a relative risk of 1.86 of developing HCC with alcohol consumption. The increased risk was present even in patients consuming a low amount of alcohol (25 g/day). There is a paucity of data comparing the clinical features of patients with HCC arising from either NAFLD or ALD. In this study, we explored differences in clinical, biochemical and tumor characteristics between NASH-HCC and ASH-HCC. This is important because there is still scant understanding regarding the natural history of NASH-HCC. As NASH is closely related to ASH, a comparison between the two etiologies will help us understand specific peculiarities that may help us manage the individual diseases better.

PATIENTS AND METHODS

Patient selection

The study cohort comprised patients in a prospective HCC database of the Department of Gastroenterology and Hepatology, Singapore General Hospital. Patients enrolled into the database from 1st July 2000 to 30th May 2013 formed the study cohort. During this period, our HCC registry enrolled 635 patients and after exclusion of patients with positive viral serology (HBsAg and HCV EIA) (n=379), non-viral HCC (n=256), remaining HCC patients (n=160), alcohol consumption (n=154), no alcohol consumption (n=66) and significant alcohol consumption (ASH-HCC) (n=45), patients were further excluded for anti-HBc positivity (n=96), patients with other etiologies of chronic liver disease (PBC, Wilson’s AIH cirrhosis) (n=6) and patients with alcohol consumption that is not significant (n=43). Metabolic risk factors were excluded (n=12). Figure 1. Patient selection flowsheet. HCC, hepatocellular carcinoma; HCV, hepatitis C virus; EIA, enzyme linked immunosorbant assay; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; ASH, alcoholic steatohepatitis; NASH, non-alcoholic steatohepatitis.
clusion criteria, 99 patients were included in the study cohort. The patients were followed till 31st October 2015 (see flowchart, Fig. 1).

Diagnosis of HCC was made based on dynamic imaging such as computerized tomography (CT) or magnetic resonance imaging (MRI). None of the patients included in the study needed a histological assessment for diagnosis of HCC.

Diagnosis of NASH-HCC was based on negative viral serology (negative HBsAg and anti-HCV IgG), by the exclusion of other liver diseases like autoimmune hepatitis induced cirrhosis, primary biliary cirrhosis, Wilson’s disease and by the absence of significant alcohol intake, in the presence of metabolic syndrome according to International Diabetes Federation and Asia Pacific Working Party on NAFLD. Our definition of NASH-HCC is similar to that of other studies in NASH-HCC.

ASH-HCC was diagnosed after negative viral serology and with significant alcohol consumption of more than 60 g/day in both genders. To exclude any effect of past hepatitis B infection on the hepatocarcinogenesis in NASH or ASH group, HBcIgG data was verified in the study population. All patients with previous hepatitis B virus (HBV) exposure as evidenced by HBcIgG positivity were excluded from the study.

There were 54 NASH-HCC and 45 ASH-HCC after consideration of inclusion and exclusion criteria. The diagnosis of cirrhosis was made on the morphological changes seen on dynamic contrast imaging of liver and/or by the presence of radiological, biochemical, endoscopic or clinical features suggestive of cirrhosis or portal hypertension.

HCC surveillance was defined as 6-monthly follow up with ultrasound imaging of the liver and serum alpha-fetoprotein (AFP) determination. Not all patients in the study were on HCC surveillance - a number of them presented with symptoms. We stratified the patients as those whose HCC were diagnosed while they were on a regular surveillance program versus those who were not on a regular surveillance program and whose HCC were diagnosed because they presented with symptoms.

**Data collection**

Demographic, anthropometric, clinical and laboratory data were collected at the time of HCC diagnosis and analyzed. Demographic data included age, gender and ethnicity. Anthropometric data included height, weight, waist circumference and body mass index (BMI). Alcohol consumption was quantified based on patients’ history and review of medical records. Clinical data reviewed included presence or absence of DM, hypertension, and hyperlipidemia. Hyperlipidemia was considered to be present in a patient with elevated triglycerides >1.7 mmol/L and reduced high density lipoprotein-C; <1.03 mmol/L in males and <1.29 mmol/L in females, or in those patients who were already taking lipid lowering medications. Liver-specific data included liver biochemistry total protein, albumin (Alb), globulin, total bilirubin (Tbil), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase, prothrombin time, international normalized ratio and full blood count including platelet count. If there was cirrhosis, Child-Turcotte-Pugh (CTP) classification and model for end-stage liver disease (MELD) score were recorded.

HCC specific data collected included modality of diagnosis, whether HCC diagnosis was made on surveillance, Barcelona Clinic Liver Cancer (BCLC) staging, and size of the tumor, multifocal or unifocal and treatment types (curative, locoregional or supportive care).

Survival outcome was based on census by our national registry of deaths on 31 October 2015. Liver related mortality was defined by death resulting from liver failure, variceal bleeding, spontaneous bacterial peritonitis or tumor rupture and tumor progression. This study was reviewed and approved by the Singapore General Hospital, Institutional Review Board.

**Statistical analysis**

Results are presented as numbers and percentages in separate columns for qualitative data or as the means and standard deviation for quantitative data. Comparisons were by 2 sample T-test for quantitative factors and Pearson’s chi-square test for qualitative factors. P values less than 0.05 from two-sided tests were considered to be significant. For survival analysis, Kaplan-Meier and Cox regression (Breslow, generalized Wilcoxon) techniques were used. All statistical analyses were performed by using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Patient characteristics**

The demographic, clinical and biochemical data are summarized in Tables 1 and 2.

The mean age at diagnosis of HCC of patients in NASH-HCC group was significantly older at 72±9 years compared to 66±9
years in the ASH-HCC group ($P<0.001$). In the NASH-HCC group there were significantly less male patients compared to the ASH-HCC group (65% vs. 96%, $P<0.001$). The ethnic composition was significantly different between the two groups with NASH-HCC having significantly more Malay patients compared to the ASH-HCC (16.7% vs. 0%) and ASH-HCC having significantly more Chinese and Indian patients compared to the NASH-HCC group (Chinese, 91.1% vs. 79.6%; Indians, 8.9% vs. 3.7%; $P<0.001$). In the NASH-HCC group, 10 patients (19%) did not have cirrhosis, whereas in the ASH-HCC group five patients (11%) did not show features of cirrhosis. This difference was not statistically significant ($P=0.3$).

The prevalence of DM, hypertension and hyperlipidemia was significantly higher in the NASH-HCC group compared to the ASH-HCC group (see Table 1). The liver biochemistry comprising Alb, Tbil, ALT, AST, and ALP were comparable between the NASH-HCC and ASH-HCC groups.

The mean BMI although higher in NASH-HCC group did not differ significantly from the ASH-HCC group. There was no difference in CTP and MELD score between the groups at the diagnosis of HCC.

Both groups showed markedly elevated mean AFP levels with a wide variability. The mean or median AFP values were not significantly different between the two groups.

**Details of HCC diagnosis**

Table 3 summarizes the aspects of HCC diagnosis. The diagnosis of HCC was made based on dynamic cross-sectional CT or MRI. A majority of the patient in both groups were diagnosed at the symptomatic stage. Symptomatic stage HCC were patients who presented with symptoms related to the HCC e.g., pain or with decompensated liver disease as opposed to those patients who were asymptomatic and had their HCC diagnosed on surveillance. In the NASH-HCC group, 56% patients were diagnosed at the symptomatic stage as compared to 53% patients in ASH-HCC group.

Around 90% patients of NASH-HCC group were not on surveillance for HCC as compared to 73% patient of ASH-HCC group. This difference was statistically significant ($P=0.046$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Etiology</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>NASH-HCC (n=54)</td>
<td>ASH-HCC (n=45)</td>
</tr>
<tr>
<td>Male</td>
<td>35 (64.8)</td>
<td>43 (95.6)</td>
</tr>
<tr>
<td>Female</td>
<td>19 (35.2)</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>NASH-HCC (n=54)</td>
<td>ASH-HCC (n=45)</td>
</tr>
<tr>
<td>Chinese</td>
<td>43 (79.6)</td>
<td>41 (91.1)</td>
</tr>
<tr>
<td>Malay</td>
<td>9 (16.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Indian</td>
<td>2 (3.7)</td>
<td>4 (8.9)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>DM</td>
<td>NASH-HCC (n=54)</td>
<td>ASH-HCC (n=45)</td>
</tr>
<tr>
<td>Yes</td>
<td>42 (77.8)</td>
<td>16 (35.6)</td>
</tr>
<tr>
<td>No</td>
<td>10 (18.5)</td>
<td>28 (62.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>43 (79.6)</td>
<td>26 (57.8)</td>
</tr>
<tr>
<td>No</td>
<td>7 (13.0)</td>
<td>18 (40.0)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>29 (53.7)</td>
<td>8 (17.7)</td>
</tr>
<tr>
<td>No</td>
<td>20 (37.0)</td>
<td>36 (80.0)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; ASH, alcoholic steatohepatitis; DM, diabetes mellitus.
Tumor characteristics

Tumour Characteristics are summarised in Table 4. There were no significant differences between the two groups in the prevalence of multifocal HCC and of portal vein invasion (see Table 4). Lymph node invasion was seen more frequently in ASH-HCC compared to NASH-HCC (22% vs. 7.5%, \( P=0.031 \)). BCLC and Tumor lymph Node Metastasis (TNM) staging were similar between the two groups. Due to the retrospective nature of study, some data regarding the TNM classification and BCLC staging was missing from our dataset.

HCC treatment

HCC treatment modalities are summarized in Table 4. Only 20% patients in ASH-HCC group received treatment with curative intent (liver transplantation, liver resection or radiofrequency ablation) compared to 26% in NASH-HCC group (\( P=0.41 \)). More than 50% of patients in both groups were not suitable for any treatment and were accorded best supportive care.

HCC survival outcomes

The survival outcome data for 96 patients (51 NASH-HCC and 45 ASH-HCC) were available. Three patients in the NASH-HCC group were foreigners who returned to their home countries after HCC diagnosis and initial treatment. Six patients (11.8%) in NASH-HCC group and three patients (6.7%) in the ASH-HCC group were not suitable for any treatment and were accorded best supportive care.

Table 3. Differences in aspects of HCC diagnosis between NASH-HCC and ASH-HCC groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Etiology</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NASH-HCC (n=54)</td>
<td>ASH-HCC (n=45)</td>
</tr>
<tr>
<td>On screening/surveillance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (11.1)</td>
<td>12 (26.7)</td>
</tr>
<tr>
<td>No</td>
<td>48 (88.9)</td>
<td>33 (73.3)</td>
</tr>
<tr>
<td>Symptomatic at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30 (55.6)</td>
<td>24 (53.3)</td>
</tr>
<tr>
<td>No</td>
<td>24 (44.4)</td>
<td>21 (46.7)</td>
</tr>
</tbody>
</table>

Values are presented as number (%). There were missing data in the registry regarding the BCLC and TNM staging. Staging data was available for 23 patients, which is presented in the table.

Table 4. Differences in the tumor characteristics between NASH-HCC and ASH-HCC groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Etiology</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NASH-HCC (n=54)</td>
<td>ASH-HCC (n=45)</td>
</tr>
<tr>
<td>Number of lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>31 (57.4)</td>
<td>26 (57.8)</td>
</tr>
<tr>
<td>Multiple</td>
<td>20 (37.0)</td>
<td>17 (37.8)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2 (3.7)</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Portal vein involvement</td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>No</td>
<td>35 (64.8)</td>
<td>28 (62.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (33.3)</td>
<td>16 (35.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.9)</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>50 (92.6)</td>
<td>34 (75.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>4 (7.4)</td>
<td>10 (22.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>BCLC stage</td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>0</td>
<td>1 (6.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>A</td>
<td>5 (31.3)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>B</td>
<td>2 (12.5)</td>
<td>1 (14.2)</td>
</tr>
<tr>
<td>C</td>
<td>2 (12.5)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>D</td>
<td>6 (37.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>TNM staging (AJCC 7ed)</td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td>I</td>
<td>9 (56.3)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>II</td>
<td>2 (12.5)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>IIIA</td>
<td>2 (12.5)</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>IIIB</td>
<td>2 (12.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IIIC</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IVA</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IVB</td>
<td>1 (6.3)</td>
<td>2 (28.6)</td>
</tr>
</tbody>
</table>

Values are presented as number (%). There were missing data in the registry regarding the BCLC and TNM staging. Staging data was available for 23 patients, which is presented in the table.

HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; ASH, alcoholic steatohepatitis; BCLC, Barcelona Clinic Liver Cancer; TNM, Tumor lymph Node Metastasis; AJCC, American Joint Committee on Cancer.
group were alive at the time of census. Survival Comparison between the groups is shown in Table 5.

Liver related mortality was defined by death resulting from liver failure, variceal bleeding, spontaneous bacterial peritonitis, tumor rupture or tumor progression. Other causes of mortality in NASH-HCC group included septicemia in five (10%), metastatic cancer of other organs in two (4%), and DM induced coma in one patients (2%). In the ASH-HCC group septicemia was cause of mortality in two (4.4%) and metastatic cancer of other organs in one patients (2.2%). Table 6 shows the other (excluding liver related) causes of mortality in both groups.

Overall survival (OS)/all-cause mortality

The median OS of NASH-HCC group was 13±2.5 months and that of ASH-HCC was 7±1.6 months (P=0.12) (Fig. 2). On multivariate logistic regressions, the significant determinant of all-cause mortality were CTP score (P<0.001) and tumor stages III (P=0.014) and IV (P<0.001). Age was not a significant factor of OS in the multivariable analysis (P=0.4), neither was presence or absence of DM, cirrhosis, portal vein or lymph node involvement (Tables 7 and 8).

Liver related mortality

With regards to liver related mortality, the median survival of NASH-HCC group was significantly better than ASH-HCC (19±10.3 vs. 8±1.3 months, P=0.047) (Fig. 3). Multivariable logistic regression showed CTP score (P=0.003) and tumor stages III (P=0.025) and IV (P=0.001) as the significant determinant of liver related mortality (Tables 7 and 8). Patient age at diagnosis, presence or absence of DM, cirrhosis, portal vein involvement or lymph node involvement were not significant determinants of liver-related mortality.

DISCUSSION

The main findings of this study are that the NASH-HCC patients were older at diagnosis, had significantly more metabolic risk factors and were diagnosed less frequently on surveillance or screening as compared to ASH-HCC.

There is often concern over the diagnosis of NASH-related HCC. NASH and metabolic syndrome are closely intertwined, where insulin resistance plays a key role in both disease entities and NASH assumed to be the hepatic manifestation of metabolic syndrome. In an earlier study of 65 patients with NASH, Chitturi et al.26

<table>
<thead>
<tr>
<th>Table 5. Survival comparison between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>NASH-HCC</td>
</tr>
<tr>
<td>ASH-HCC</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Values are presented as number (%). N, total number of patients; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; ASH, alcoholic steatohepatitis.

<table>
<thead>
<tr>
<th>Table 6. Causes of non-liver-related mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
</tr>
<tr>
<td>NASH-HCC (9/51)</td>
</tr>
<tr>
<td>Septicemia</td>
</tr>
<tr>
<td>Other metastatic cancers (colon, 1; unknown primary, 1)</td>
</tr>
<tr>
<td>DM coma</td>
</tr>
<tr>
<td>Multi-organ failure of unknown cause</td>
</tr>
<tr>
<td>ASH-HCC (4/45)</td>
</tr>
<tr>
<td>Septicemia</td>
</tr>
<tr>
<td>Other metastatic cancers (prostate, 1)</td>
</tr>
<tr>
<td>Multi-organ failure of unknown cause</td>
</tr>
</tbody>
</table>

Values are presented as number (%). NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; DM, diabetes mellitus; ASH, alcoholic steatohepatitis.
<table>
<thead>
<tr>
<th>Variable</th>
<th>All-cause mortality</th>
<th>Liver-related mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Etiology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NASH-HCC</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>ASH-HCC</td>
<td>1.31 (0.94–1.68)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>1.01 (0.99–1.04)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.40 (0.83–2.36)</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>DM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.14 (0.74–1.74)</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>HTN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.21 (0.76–1.91)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>HLD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.86 (0.56–1.33)</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Cirrhosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.77 (0.49–1.22)</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>0.93 (0.90–0.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ALP</strong></td>
<td>1.005 (1.003–1.007)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>1.001 (0.99–1.01)</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>1.007 (1.004–1.009)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>GGT</strong></td>
<td>1.001 (1.0009–1.002)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>AFP</strong></td>
<td>1.000003 (1.000001–1.000006)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Total bilirubin</strong></td>
<td>1.005 (1.002–1.009)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Serum creatinine</strong></td>
<td>1.003 (1.001–1.005)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>MELD score</strong></td>
<td>1.05 (1.004–1.010)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>CTP score</strong></td>
<td>1.29 (1.16–1.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Portal vein involved</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.35 (2.08–5.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Lymph node involved</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4.35 (2.29–8.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>TNM staging (AJCC 7th ed)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.88 (1.004–3.52)</td>
<td>0.05</td>
</tr>
<tr>
<td>III</td>
<td>3.00 (1.75–5.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV</td>
<td>8.58 (4.08–18.03)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; ASH, alcoholic steatohepatitis; DM, diabetes mellitus; HTN, hypertension; HLD, hyperlipidaemia; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma glutamyl transferases; AFP, alpha-fetoprotein; MELD, model for end-stage liver disease; CTP, Child-Turcotte-Pugh; TNM, Tumor lymph Node Metastasis; AJCC, American Joint Committee on Cancer.

showed that virtually all subjects (98%) were insulin resistant. It is commonly accepted that in the context of liver disease, presence of metabolic syndrome connotes presence of NASH, in the absence of other etiologies of chronic liver disease, such as alcohol or viral related causes. Separately, Bugianesi et al. demonstrated that features suggestive of the metabolic syndrome, such as DM, dyslipidemia, obesity and insulin resistance were reported more frequently in patients with HCC arising in the setting of cryptogenic/NASH cirrhosis than in matched controls.

We used a definition of NASH-HCC based on the above considerations and which is similar that used in other studies on NASH HCC.20,21 The biochemical parameters were not significantly different between the groups. Interestingly a number of patients in NASH-HCC (18.5%) and ASH-HCC (11.1%) were not cirrhotic at diagnosis of HCC. Since many NASH-HCC patients were diagnosed at symptomatic stage and not by surveillance or screening a more rigorous screening and surveillance methods is required for NASH-HCC patients than currently practiced. In terms of overall mortality both the groups were the same but NASH-HCC patients fared better than ASH-HCC in terms of liver related mortality. Our study also showed that significantly less NASH-HCC patients die from liver-related mortality compared to ASH-HCC. Instead, more NASH-HCC patients also died from septicemia unrelated to liver cancer, diabetes induced coma and metastatic disease of other organs as compared to ASH-HCC group.

Even though histologically NASH and ASH are quite similar they are different in the natural history and liver-related complication rates.28,29 ASH and NASH can be considered as diseases with differing etiologies but of a similar morphologic spectrum. One of the main complications of both NASH and ASH is the development of HCC.

### Table 8. Multivariable Cox regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>All-cause mortality</th>
<th>Liver-related mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Etiology</td>
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<td></td>
</tr>
<tr>
<td>NASH-HCC</td>
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<td></td>
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<tr>
<td>ASH-HCC</td>
<td>1.28 (0.73–1.87)</td>
<td>0.08</td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (0.9995–1.06)</td>
<td>0.40</td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
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</tr>
<tr>
<td>Yes</td>
<td>0.91 (0.55–1.50)</td>
<td>0.70</td>
</tr>
<tr>
<td>Cirrhosis</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Yes</td>
<td>0.74 (0.43–1.28)</td>
<td>0.28</td>
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<tr>
<td>CTP</td>
<td>1.28 (1.12–1.46)</td>
<td>&lt; 0.001</td>
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<td>Portal vein involved</td>
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<td></td>
</tr>
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<td>1.00</td>
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</tr>
<tr>
<td>Yes</td>
<td>1.47 (0.78–2.76)</td>
<td>0.23</td>
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<td>Lymph node involved</td>
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<td></td>
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<td>1.07 (0.47–2.41)</td>
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<td>TNM staging (AJCC 7th ed)</td>
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</tr>
<tr>
<td>I</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2.00 (0.96–4.16)</td>
<td>0.06</td>
</tr>
<tr>
<td>III</td>
<td>2.31 (1.19–4.51)</td>
<td>0.01</td>
</tr>
<tr>
<td>IV</td>
<td>6.38 (2.19–18.57)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; ASH, alcoholic steatohepatitis; DM, diabetes mellitus; CTP, Child-Turcotte-Pugh; TNM, Tumor lymph Node Metastasis; AJCC, American Joint Committee on Cancer.
The liver undergoes simultaneous regeneration and fibrosis in response to injury. These divergent responses lead to distinctive pathways of hepatocarcinogenesis. Potential mechanisms of fibrosis-dependent carcinogenesis include increased integrin signalling by the fibrotic matrix, paracrine signalling between hepatic stellate cells and hepatocytes, increased stromal stiffness, growth factor sequestration by extracellular matrix, and reduced tumor surveillance by natural killer and natural killer T cells. Apart from the fibrosis induced hepatocarcinogenesis, chronic alcohol consumption interferes with several host anti-tumor mechanisms, thereby facilitating hepatocyte proliferation and tumorigenesis. The major mechanisms of alcohol-induced HCC include pathways of ethanol metabolism, alcohol-induced oxidative stress and hypomethylation of DNA, and interplay of alcohol with iron elevation, retinoid metabolism, the immune system, inflammatory pathways, and neoangiogenesis. The understanding of hepatocarcinogenesis in NASH although still evolving, is believed to originate from deregulated one-carbon metabolism, nuclear factor kB proteins, phosphatase and tensin homolog (PTEN), microRNA instability and telomere shortening.

Consistent with published literature our patients with NASH-HCC were significantly older than patients with ASH-HCC with most of the patients in NASH-HCC group in their 7th decade of life whereas patients in ASH-HCC group were a decade younger. Older age at diagnosis of NASH-HCC cohort has clinical implications. HCC screening and surveillance strategies should include elderly patients and the treatment strategies should also address treatment approaches in elderly patients. NASH-HCC group comprised significantly more female patients compared to the ASH-HCC group, which is consistent with the alcohol consumption pattern worldwide. Interestingly our study showed that 18% of the patients in NASH-HCC group and 11% of the patients in ASH-HCC group had no apparent signs of cirrhosis on radiological examination. This observation echoes previously published literature demonstrating the occurrence of NASH related HCC in the absence of cirrhosis. There is an increasing body of evidence showing that HCC can occur in patients with non-cirrhotic NASH. Two recent large studies from Japan of 292 and 87 patients with NAFLD and HCC reported that non-cirrhotic patients comprised 38% and 49% respectively of the cases of HCC. A recent meta-analysis of 168,571 subjects revealed that non-cirrhotic NASH subjects were 2.61 times significantly more likely to develop HCC compared to non-cirrhotic subjects of other etiologies. Another study from the USA reported 34.6% of patients in NAFLD-HCC group and 11.1% of ALD-related HCC as not having cirrhosis at diagnosis. This is consistent with the results of our study. One possible reason for HCC occurring in non-cirrhotic ASH is that the main underlying mechanism for hepatocarcinogenesis in ALD is the oxidative stress of chronic inflammation coupled with induction of cytochrome P-450 by alcohol leading to reactive oxygen species production that in turn causes lipid peroxidation, DNA damage, iron overload and immune system dysfunction. This hepatocarcinogenesis pathway is independent of fibrosis and cirrhosis. Nevertheless, the development of HCC in ASH/ALD without cirrhosis may not be widely recognised and warrants further studies.

Another notable observation in our study was that majority of patients in both groups were symptomatic at diagnosis. Only 11.1% of the patients in NASH-HCC group were diagnosed on surveillance imaging compared to 26.7% of the patients in ASH-HCC group. This finding underlines the fact that NASH patients may not be receiving adequate surveillance for liver-related complications since there is no consensus regarding HCC surveillance in NASH. Major society guidelines recommend 6 monthly ultrasonography surveillance for HCC on at-risk population, i.e., in cirrhotic patients and HBV positive patients in endemic areas. Considering the fact that approximately 20% of patients with NASH develop HCC in a non-cirrhosis setting, which is also corroborated by the data of our study, where 18% of patients in NASH-HCC group were non-cirrhotic, is a need to identify these NASH patients who are at increased risk of developing HCC so
that appropriate surveillance strategies can be formulated. Currently, this subset of the patient is poorly defined.

To reduce the morbidity and mortality associated with late detection of HCC, we propose that patients with ALD as well as those with NAFLD/NASH should have fibrosis assessment and those with advanced/bridging fibrosis (>F3) or cirrhosis should be on 6-monthly surveillance for HCC. This strategy is echoed in the recently published HCC guidelines from the European Association of study of liver diseases. \(^{37}\) Individualized intensive screening programs based on risk prediction of HCC like ADDRESS-HCC\(^{10}\) needs further validation in different cohorts.

Approximately 40% of the patients in both groups had multifocal or diffuse HCC. Portal vein involvement and tumor extent were similar between the NASH-HCC and ASH-HCC groups. Majority of the patients in both groups were diagnosed late in the course of disease and more than 50% of them were symptomatic at presentation. As such, treatment options were limited in many patients. Only 4.4% patients in the ASH-HCC group and 13% patients in the NASH-HCC group underwent curative resection. None of the patients in either group could receive liver transplantation due to advanced disease and non-availability of a liver graft. Similar experiences in the treatment of NASH-HCC were reported by Weinmann and colleagues,\(^{49}\) who in a cohort of 45 biopsy proven NASH-HCC patients found only 17.8% went for resection, 4.4% for liver transplant after bridging therapy and 73.3% for locoregional therapy or supportive care. Recently data from a large French cohort consisting of 582 alcohol induced HCC reported that treatment with curative intent was less likely to happen in alcohol induced HCC, only 16.3% patient could undergo treatment with curative intent.\(^{50}\)

Our study showed that for all-cause mortality, although the median OS of NASH-HCC was numerically better than that of ASH-HCC (13 months vs. 7 months), it was not statistically significant \((P=0.12)\). However, the median survival of NASH-HCC group was significantly better than ASH-HCC (19 months vs. 8 months) for liver related mortality \((P=0.047)\). Patient’s age at diagnosis of HCC did not affect the median OS or liver-related mortality of both groups. This etiological difference of survival between the groups is consistent with previously published NASH-HCC survival data from Weinmann and colleagues\(^{49}\) and alcohol induced HCC survival data by Costentin and colleagues\(^{50}\) who reported the lead time adjusted median OS of 5.7 months in 528 alcohol induced HCC patients.

The reason for difference in liver related mortality between the groups, with the NASH-HCC group having significantly better survival, is that the alcohol related HCC patients were likely to be imbibing alcohol till the point of admission, with a fair number of them having florid alcoholic hepatitis which played an important part in their liver related mortality. However, the OS which was not statistically different between the two groups (although showing a better trend for NASH-HCC group) was due to the fact that after the initial period, it’s the tumour stage and grade that were predominant factors in determining the mortality.

Our study is limited by its retrospective nature and single center data. Alcohol quantification was via patient self-reporting and electronic medical records, which cannot exclude a certain degree of misclassification. Nevertheless, this study provides important information on HCC attributed to two well established etiologies of chronic liver disease in an Asian centre. It is the first study to compare the difference in demographics, clinical, biochemical, tumor characteristics and survival outcomes between NASH and ASH related HCC. Data collection through interconnected electronic record system and mandatory national mortality reporting makes our data robust. Further prospective studies should look for ways to improve OS in both groups.

NASH-HCC and ASH-HCC differ in their etiology and pathogenesis. Despite these differences, liver and tumor characteristics were comparable between NASH-HCC and ASH-HCC. Patients with NASH-HCC had more metabolic risk factors and were older at diagnosis compared to ASH-HCC. Most patients in both groups were diagnosed late and were not amenable to curative or loco-regional therapies. OS of NASH-HCC and ASH-HCC were not statistically different. Better characterization of the at-risk population of patients with NASH and ASH are needed to optimize screening, surveillance, and management of NASH and ASH related HCC.

Authors’ contribution
RK, BBGG and CKT conceptualised the study, collected and analysed the data and wrote the manuscript, JWK performed detailed statistical analyses of the data, PEC collected and analysed the data, reviewed and refined the manuscript.

Acknowledgements
The authors wish to acknowledge all the doctors in the Department of Gastroenterology and Hepatology, Singapore General Hospital, for allowing their patients to be enrolled into the HCC registry.
Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

7. Albhai S, Sanyal A. Recent advances in understanding and managing non-alcoholic fatty liver disease. F1000Res 2018;7(F1000 Faculty Rev):720.
Trends in the prevalence of chronic liver disease in the Korean adult population, 1998–2017

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Graphical Abstract

The prevalence of NAFLD and ALD increased from 18.6% to 21.5% and from 3.8% to 7.0%, respectively, between 1998–2001 and 2016–2017.

There was a downward trend in CHB with a current overall prevalence near 3.4%.

Abbreviations:
ALD, alcohol-related liver disease; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CI, confidence interval; CLD, chronic liver disease; FIB-4, fibrosis-4; HCV, hepatitis C virus; KNHANES, Korea National Health and Nutrition Examination Survey; NAFLD, non-alcoholic fatty liver disease; WHO, world health organization

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Editor: Ju Hyun Shim, University of Ulsan College of Medicine, Korea

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INTRODUCTION

According to global health estimates 2015 of the World Health Organization (WHO), approximately 1.2 million people worldwide die each year from cirrhosis and subsequent complications and 800,000 die of hepatocellular carcinoma, comprising 3.5% of all deaths worldwide.\textsuperscript{1,2} Especially, the Asia-Pacific region accounts for 45% of the world's deaths due to liver disease. As chronic liver disease (CLD) may develop due to a variety of etiologies including chronic viral infection, alcohol, and metabolic causes, the implementation of population-based interventions that screen for the underlying cause and the use of lifestyle and pharmacological interventions to prevent or delay the progression to life-threatening cirrhosis complications and liver cancer is imperative.

The WHO recently proposed a strategic plan consisting of global efforts to eradicate hepatitis virus, the main cause of liver disease.\textsuperscript{3} Despite ongoing efforts, the global burden of CLD is anticipated to increase in a generation.\textsuperscript{4} More than 5% of all deaths worldwide are attributed to the harmful use of alcohol, and alcohol-related liver disease (ALD) accounts for one quarter of all alcohol-related deaths.\textsuperscript{4} Considering the increase in per capita alcohol consumption in the Asia-Pacific region,\textsuperscript{5} the prevalence of ALD and its complications is expected to increase further in the near future. Non-alcoholic fatty liver disease (NAFLD), the most notable liver disease, has emerged as a new management subject as viral liver disease enters a genuine phase.\textsuperscript{6} NAFLD is also expected to increase as the incidence of obesity increase due to westernized eating habits, a lack of exercise, and lifestyle changes.\textsuperscript{7}

Over the past three decades, there have been great successes in CLD management, particularly in viral hepatitis, as well as epidemiological changes. However, data on trends in the prevalence of CLD in Korea are sparse. Recently, NAFLD modeling is being actively attempted to forecast the disease burden using currently available data.\textsuperscript{7} A nationwide prevalence investigation would provide more accurate rates in entire population and specific subpopulations\textsuperscript{8} for use as basic data for CLD modeling.

Accordingly, we sought to determine the current prevalence of CLD in Korean adults aged 19 and older and whether the prevalence changed between 1998–2001 and 2016–2017 in a representative Korean adult population data from the Korea National Background/Aims: Data on the trends in the prevalence of chronic liver disease (CLD) in Korea are scarce. This study aimed to evaluate whether the CLD prevalence changed between 1998–2001 and 2016–2017.

Methods: Data were extracted from the Korea National Health and Nutrition Examination Survey (1998–2001 to 2016–2017; n=25,893). Non-alcoholic fatty liver disease (NAFLD) was defined as a hepatic steatosis index >36 in the absence of any other evidence of CLD. The definition of alcohol-related liver disease (ALD) was excessive alcohol consumption (≥210 g/week for men and ≥140 g/week for women) and an ALD/NAFLD index >0.

Results: The prevalence of NAFLD increased from 18.6% (95% confidence interval [CI], 17.8–19.5%) in 1998–2001 to 21.5% (95% CI, 20.6–22.6%) in 2016–2017. During the same time period, increases were observed in the prevalence of obesity (27.0 vs. 35.1%), central obesity (29.4 vs. 36.0%), diabetes (7.5 vs. 10.6%), and excessive drinking (7.3 vs. 10.5%). ALD prevalence also increased from 3.8% (95% CI, 3.4–4.2%) to 7.0% (95% CI, 6.4–7.6%). In contrast, chronic hepatitis B decreased from 5.1% (95% CI, 4.6–5.5%) to 3.4% (95% CI, 3.0–3.8%). The prevalence of chronic hepatitis C was approximately 0.3% in 2016–2017.

Conclusions: The prevalence of NAFLD and ALD increase among Korean adults. Our results suggest potential targets for interventions to reduce the future burden of CLD. (Clin Mol Hepatol 2020;26:209-215)

Keywords: Alcoholic-related liver disease; Hepatitis B, Chronic; Hepatitis C, Chronic; Non-alcoholic fatty liver disease; Prevalence
MATERIALS AND METHODS

Sample population

The KNHANES is a series of cross-sectional national health and nutrition surveys designed to provide representative prevalence estimates for a variety of health measures and conditions. The KNHANES is conducted by the Korean Centers for Disease Control. The survey design is a complex, stratified, multi-stage probability sampling of the civilian, non-institutionalized Korean population. The procedures used to select the sample as well as to conduct the interviews and examinations have been specified elsewhere. This survey included an interview to obtain information concerning an individual’s health history, health behaviors, and risk factors. A subsequent health examination was performed at a mobile examination center.

Our analyses included data from 1998–2001 (KNHANES I and II) and 2016–2017 (KNHANES VII-1 and VII-2). We included a total of 14,801 participants aged 19 years and older from KNHANES 1998–2001 and 12,900 participants from KNHANES 2016–2017, respectively in this study. Of that number, 1,808 subjects who had incomplete data on NAFLD prediction model or biochemical/clinical parameters were excluded. Finally, 25,893 (14,438 in KNHANES 1998–2001 and 11,455 in KNHANES 2016–2017) participants were included in the analyses. Informed consent was obtained from all participants, and the protocol was approved by the Institutional Review Board of the Korean Centers for Disease Control.

Definition of CLD etiology

NAFLD was defined using the validated hepatic steatosis index which was calculated as $8 \times \text{alanine aminotransferase} / \text{aspartate aminotransferase (ALT/AST)} + \text{body mass index (BMI)} (+2, if diabetes; +2, if female)$ in the absence of any other evidence of CLD, such as excessive alcohol intake or a positive hepatitis B or hepatitis C test. From 1998–2001, since anti-hepatitis C virus (anti-HCV) tests were not performed in KNHANES, chronic hepatitis C (CHC) cannot be ruled out in defining NAFLD during that period. The optimal cut-off value for NAFLD was set at >36. In the subpopulation with NAFLD, the AST-to-platelet ratio index (APRI)$^{11}$ and fibrosis-4 (FIB-4)$^{12}$ were used to evaluate liver fibrosis. Their formula were as follows: $\text{APRI} = (\text{AST} / \text{upper limit of normal}) / (\text{platelet count} \times 10^{-9}) \times 100$, where the upper limits of normal AST levels were set at 37 IU/L for men and 29 IU/L for women; $^{13}$ $\text{FIB-4} = \text{age} \times \text{AST} / (\text{platelets in} \times (\text{ALT})^{1/2})$. Cut-off values for advanced fibrosis ($\geq F3$) were set at 1 of APRI and 2.67 of FIB-4. APRI and FIB-4 were calculated only in KNHANES 2016–2017 due to a lack of data in the KNHANES 1998–2001.

Chronic hepatitis B (CHB) was defined as HBsAg seropositivity. An anti-HCV test was done only in the KNHANES 2016–2017, but an HCV RNA polymerase chain reaction (PCR) test was not performed. We assumed that CHC affected approximately 30% of those with anti-HCV positivity based on an observation in KNHANES 2013–2015, in which an HCV RNA PCR test showed that 37 individuals were HCV RNA positive among 119 subjects with anti-HCV positivity. The study definition of ALD was a com-

Table 1. Demographic and clinical characteristics of the study sample

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Total</td>
<td>14,438</td>
<td>11,455</td>
<td></td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Smoking (%)</td>
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<td>Excessive drinking (%)</td>
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<td>Obesity (%)</td>
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<td>Central obesity (%)</td>
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<tr>
<td>Diabetes (%)</td>
<td>7.5</td>
<td>10.6</td>
<td>&lt;0.001</td>
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<tr>
<td>Hypertension (%)</td>
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<td>TyG index</td>
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<td>4.65</td>
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<td>Chronic hepatitis C (%)</td>
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</table>

Values are presented as weighted percentages or means. Excessive drinking: $\geq 210$ g/week for men and $\geq 140$ g/week for women; obesity: body mass index $\geq 25$; central obesity: waist circumference $\geq 90$ cm for men and $\geq 80$ cm for women. TyG index, triglycerides and glucose index; NAFLD, non-alcoholic fatty liver disease.
Combination of excessive alcohol consumption (≥210 g/week for men and ≥140 g/week for women) and an ALD/NAFLD index >0, which was calculated as −58.5 + 0.637 (mean corpuscular volume) + 3.91 (AST/ALT) − 0.406 (BMI) + 6.35 for male gender.14

Definition of clinical parameters

Diabetes mellitus was defined based on use insulin or oral hypoglycemic agents or fasting plasma glucose ≥126 mg/dL. Participants were diagnosed as hypertensive if the systolic pressure was ≥140 mmHg, diastolic pressure was ≥90 mmHg, or antihypertensive medication was used. Hypercholesterolemia was defined as a cholesterol level ≥240 mg/dL. Obesity was defined as a BMI of ≥25,15 while central obesity was defined using the thresholds for waist circumference (≥90 cm for men and ≥80 cm for women).16

Insulin resistance was calculated using the triglycerides and glucose index as follows: Ln [fasting glucose (mg/dL) × triglycerides (mg/dL) / 2].17

Data analyses

Sample weights were included in the estimation process for all of the analyses to reflect the differential probabilities for selection, non-response, and non-coverage.9 All analyses were performed using Complex Samples in SPSS statistics (version 25.0; IBM Corp., Armonk, NY, USA), which provides the specialized statistics for complex sample designs, such as stratified, clustered or

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>NAFLD Male</td>
<td>18.7</td>
<td>23.7</td>
</tr>
<tr>
<td>Female</td>
<td>18.6</td>
<td>19.3</td>
</tr>
<tr>
<td>Alcohol-related liver disease Male</td>
<td>7.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Female</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Chronic hepatitis B Male</td>
<td>5.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Female</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Chronic hepatitis C Male</td>
<td>NA</td>
<td>0.4</td>
</tr>
<tr>
<td>Female</td>
<td>NA</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values are presented as weighted percentages.

NAFLD, non-alcoholic fatty liver disease.

Figure 1. Prevalence of CLD by age groups on KNHANES in 1998–2001 and 2016–2017. Data are reported as weighted percentages. (A) NAFLD prevalence, (B) ALD prevalence, (C) CHB prevalence. CLD, chronic liver disease; NAFLD, non-alcoholic fatty liver disease; ALD, alcohol-related liver disease; CHB, chronic hepatitis B.
multistage sampling. When merging NHANES survey cycles, appropriate selection of sampling weights and adjustment coefficients were applied according to the NHANES guidelines. The data were presented as weighted means or weighted proportions with standard errors for continuous or categorical variables, respectively. Differences between the two study cycles were examined with Wald F test statistics in a general linear model for continuous variables and the Rao-Scott adjusted chi-square test for categorical variables.

RESULTS

Table 1 shows the weighted distribution of the NHANES 1998–2001 and 2016–2017 samples. With the exception of smoking status and CHB, all clinical parameters disadvantageously changed during two decades. Compared with 1998–2001, the prevalence in 2016–2017 increased for obesity, diabetes, and hypertension. These unfavorable changes represented with an increased NAFLD prevalence in 2016–2017 from 18.6% (95% confidence interval [CI], 17.8–19.5%) in 1998–2001 to 21.5% (95% CI, 20.6–22.6%). Along with increase in excessive drinking, the prevalence of ALD also increased from 3.8% (95% CI, 3.4–4.2%) in 1998–2001 to 7.0% (95% CI, 6.4–7.6%) in 2016–2017. In contrast, the prevalence of CHB in 2016–2017 decreased from 5.1% (95% CI, 4.6–5.5%) in 1998–2001 to 3.4% (95% CI, 3.0–3.8%) in 2016–2017. The prevalence of CHC was approximately 0.3% in 2016–2017. Gender-specific trends in the prevalence of CLD are presented in Table 2. Changes in the prevalence of NAFLD, ALD, and CHB during the study period were evident in both genders.

Figure 1 shows the changes in the prevalence of NAFLD, ALD, and CHB. The increase in the prevalence of NAFLD was led by the 20s, 30s, and 60s age groups. With the exception of the 60s group, increases in ALD prevalence were seen in all age strata. There was a sharp increase in ALD prevalence in 20s from 1.6% (95% CI, 1.1–2.3%) in 1998–2001 to 6.4% (95% CI, 4.9–8.3%) in 2016–2017. The prevalence of CHB was decreased in all age groups except in the 60s. In particular, the prevalence decreased dramatically in the 20s from 5.0% (95% CI, 4.1–6.2%) in 1998–2001 to 1.2% (95% CI, 0.7–2.0%) in 2016–2017. A similar trend was observed in the 30s from 5.8% (95% CI, 5.1–6.7%) in 1998–2001 to 3.6% (95% CI, 2.7–4.8%) in 2016–2017.

When the study sample was stratified by selected characteristics, the prevalence of NAFLD was approximately 50% among participants with obesity and diabetes and 30% among those with hypertension and hypercholesterolemia in both survey cycles (Table 3). Among the 2,440 participants with NAFLD in 2016–2017, the prevalence of advanced fibrosis, defined by an APRI >1 and an FIB-4 >2.67, were 1.4% (95% CI, 0.9–2.1%) and 1.2% (95% CI, 0.8–1.8%), respectively.

DISCUSSION

The current study provides an estimate of the current and past prevalence of CLD in Korean adult population and demonstrates how the prevalence has changed over two decades using NHANES surveys. The NHANES is the only population-based survey that provides nationally representative estimates of the prevalence- and, therefore, the lifetime risk- of CLD. Consequently, relatively accurate, clinically relevant population estimates can be generated, and time trends can be identified. The two surveys described here indicated that the prevalence of NAFLD and ALD increased by 16% and 84%, respectively, in persons aged 19 years or older over the 19-year interval. In contrary, we found encouraging results with regard to the prevalence of CHB which decreased by 33%.

From our analyses, we estimate that the prevalence of NAFLD increased from 18.6% in 1998–2001 to 21.5% in 2016–2017.

Table 3. Prevalence of NAFLD by selected characteristics

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td>Obesity</td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>5.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Yes</td>
<td>54.2</td>
<td>53.7</td>
</tr>
<tr>
<td>Central obesity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Yes</td>
<td>44.7</td>
<td>48.4</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16.4</td>
<td>18.3</td>
</tr>
<tr>
<td>Yes</td>
<td>46.0</td>
<td>49.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15.8</td>
<td>17.9</td>
</tr>
<tr>
<td>Yes</td>
<td>28.3</td>
<td>31.5</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17.0</td>
<td>18.7</td>
</tr>
<tr>
<td>Yes</td>
<td>36.8</td>
<td>32.8</td>
</tr>
</tbody>
</table>

Values are presented as weighted percentages.
NAFLD, non-alcoholic fatty liver disease.
This increase was evident in age groups of 20s, 30s, and 60s as well as in both genders. This increase may be large due to the increasing population of obesity and diabetes in which the prevalence of NAFLD is approximately 50%. It also could be attributable to the aging Korean population (42 years in 1998–2001 vs. 47 years in 2016–2017). This increasing trend of NAFLD prevalence reported here is similar to that of a recent systematic review of an Asian population. Another study predicted that NAFLD prevalence in Asia will increase from 18% in 2016 to 22% by 2030 and that obesity will increase from ~27% in 2016 to 28.5% by 2030. Taken together, our results demonstrate that unless there is apparent attenuation in the trend of well-established risk factors, the prevalence of NAFLD is expected to continually increase. We also found that 1.2–1.5% of the participants with NAFLD had advanced fibrosis (≥F3). Its prevalence is similar to previously reported estimates in US population, representing a major disease burden in now and near future.

As expected by the observed increase in excessive alcohol consumption, the estimated prevalence of ALD increased approximately 85% over this same period. This sharp increasing trend in ALD was primarily driven by increasing ALD levels in young adults. Our analysis identified a strong and upward trend in ALD prevalence among person younger than 40 years. Considering that the early initiation of alcohol abuse in early adulthood is associated with unhealthy drinking patterns and adverse psychosocial, behavioral, and other long-term health outcomes, this unfavorable trend of excessive consumption corresponding with ALD in young adults is a concern from a public health perspective.

Our results shed some light on encouraging trends in CHB. We observed a downward trend in CHB across all age groups, with the exception of the 60s. In the 20s age group, we revealed that the prevalence of CHB fell from 5% in 1998–2001 to 1.2% in 2016–2017. Because CHB is a lifetime infectious disease, any increases or decreases in CHB are expected to be first evident in younger persons. This finding is in agreement with a previous study, which reported that the incidence and prevalence of CHB decreased over the last two decades due to the successful implementation of a vaccination program. Despite the substantial progress in preventing CHB in young adults, this study confirms the ongoing high prevalence of CHB among Korean adults. The consequent burden of CHB remains high and is consistent with findings from a recent modelling study.

Our findings of the prevalence of anti-HCV positivity (0.8%; 95% CI, 0.6–1.0%) are consistent with a prior analysis of Korean data in 2009 that reported 0.8% of about 300,000 health-check examinees. Our study extended the temporal trend analysis through 2017 to demonstrate continued stability of anti-HCV positivity. Based on a finding that one third resulted in HCV RNA positivity among those with anti-HCV positivity, the extrapolation of KNHANES data suggests a 0.3% CHC prevalence in 2016–2017. The estimated prevalence of CHC reported here is much lower than previous estimates (approximately 1%) in Korea or a recent global estimate, which was based on anti-HCV positivity.

The main strength of the current study is that the data were representative of the Korean population and collected using standardized measurements. Although this study makes important contributions to the literature, it also has a number of limitations. First, because of the absence of imaging or histological diagnosis of NAFLD and ALD, we adopted operational criteria for defining NAFLD and ALD based on a predictive model that has been validated. Therefore, interpretation of our results should be made with caution. Second, the data on alcohol measurement were based on self-reports; thus, they may be influenced by recall and social desirability bias. Third, rather than tracing the changes that occurred over decades, our study simply compared the prevalence at two time points. Although we can confirm an increase in NAFLD and ALD during the study period, it is unclear whether the prevalence of the two diseases was continuously increasing. Finally, the survey participants were sampled only from the non-institutionalized Korean population. The following persons were excluded from the analysis: incarcerated persons, homeless persons, and person who were physically unable to undergo the survey. Therefore, the overall CLD prevalence might be underestimated.

In conclusion, our study found that the prevalence of NAFLD and ALD, particularly in young adults, increased in the Korean adult population from 18.6% to 21.5% and from 3.8% to 7.0%, respectively, between 1998–2001 and 2016–2017. However, there is a downward trend in CHB with a current overall prevalence near 3.4%. The CHC prevalence appears to have plateaued 0.3%. Our results demonstrate potential targets for interventions to reduce the future burden of CLD. Public health efforts are required to prevent and improve modifiable risk factors of CLD.

Author’s contribution
**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


Limited expression of TLR9 on T cells and its functional consequences in patients with nonalcoholic fatty liver disease

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INTRODUCTION

Nonalcoholic fatty liver diseases (NAFLD) are a spectrum of liver diseases including simple steatosis (SS) and steatohepatitis as its more progressive form.1 The hallmark of nonalcoholic steatohepatitis (NASH) is the intrahepatic recruitment of inflammatory cells. All cells able to produce interferon gamma (IFN-γ) in inflammation can regulate the polarization of macrophages towards a pro-inflammatory phenotype. Compared with NASH, SS patients exhibit higher numbers of intrahepatic macrophages of anti-inflammatory nature.2

Toll-like receptors (TLR) are a family of pattern recognition receptors able to activate the innate immune system.3 TLR9-mediated triggering of hepatic Kupffer cells is critically involved in NASH development4 firstly because NAFLD-associated small intestinal bacterial overgrowth2,3 promotes the entry of TLR9 ligands of bacterial origin into the liver5 and secondly, due to the presence of microparticles of hepatocyte origin carrying mitochondrial DNA which also functions as TLR9 ligands.6

T cell responses in diseases of diverse etiology are modulated

Abbreviations:
BMI, body mass index; CpG-ODN, cytidine-phosphate-guanosine oligodeoxynucleotide; FITC, fluorescein isothiocyanate; IFN-γ, interferon gamma; mAb, monoclonal antibody; MFI, mean fluorescence intensity; NAFLD, nonalcoholic fatty liver diseases; NASH, nonalcoholic steatohepatitis; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; PerCP, Peridinin-Chlorophyll-protein; SS, simple steatosis; TIR, toll-interleukin 1 receptor; TLR, toll-like receptors

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Editor: Sang Gyune Kim, Soonchunhyang University College of Medicine, Korea

Received: Aug. 8, 2019 / Revised: Oct. 2, 2019 / Accepted: Oct. 22, 2019

Background/Aims: Toll-like receptors (TLRs) modulate T cell responses in diverse diseases. Co-stimulation of T cell activation via TLR9 induces production of interferon gamma (IFN-γ), priming of which is critical for differentiation of pro-inflammatory macrophages. These macrophages have a crucial role in nonalcoholic fatty liver disease (NAFLD). We aimed to evaluate the expression of TLR9 protein on T cells and the consequences of TLR9-mediated triggering of these cells in patients with NAFLD.

Methods: Our study included 34 patients with simple steatosis, 34 patients with nonalcoholic steatohepatitis, eight patients with NAFLD who met general diagnostic criteria but lacked histological diagnosis, and 51 control subjects. We used a synthetic TLR9 ligand to co-stimulate T cells. We measured TLR9 expression in liver and peripheral T cells and CD69 and IFN-γ as phenotypic markers of T cell activation and differentiation by flow cytometry.

Results: TLR9 expression on liver and peripheral T cells was lowest in patients with simple steatosis and was positively associated with anthropometric, biochemical, and histopathological features of NAFLD. In vitro co-stimulation of T cells from patients with simple steatosis induced a limited number of IFN-γ-producing CD8+ T cells. At baseline, these patients showed a low frequency of circulating type 1 CD8+ cells.

Conclusions: The positive associations between TLR9 and anthropometric, clinical, and histological features and the crucial role of IFN-γ in NAFLD suggest that limited TLR9 expression and production of IFN-γ play a protective role in patients with simple steatosis. (Clin Mol Hepatol 2020;26:216-226)

Keywords: Humans; Non-alcoholic fatty liver disease; CD8-positive T-lymphocytes; Toll-like receptor 9

Study Highlights
We demonstrated a limited expression of toll-like receptor 9 (TLR9) and interferon gamma (IFN-γ) production by T cells in patients with simple steatosis (SS). The limited expression of TLR9 on T cells was directly associated with lower liver necroinflammatory activity and fibrosis, and lower anthropometric and biochemical alterations of nonalcoholic fatty liver disease. Besides, co-stimulation of T cell activation via TLR9 induced a limited number of IFN-γ-producing CD8+ T cells in SS patients. Accordingly, these patients showed a low frequency of circulating type 1 CD8+ cells whereas additional pro-inflammatory signals may be responsible for the higher frequency observed at baseline in the context of steatohepatitis.
by TLRs expression,\textsuperscript{7,11} including TLR9.\textsuperscript{13} T cells co-stimulation via T cell receptor and TLR9 causes T cell activation and IFN-\(\gamma\) production.\textsuperscript{12} We have previously identified the alteration of different T cell subsets in NASH patients\textsuperscript{14} and other authors confirmed a key role for T cells in NASH.\textsuperscript{15} A low intrahepatic expression of TLR9 mRNA was reported in SS.\textsuperscript{15} though it may probably come from the liver parenchyma than from T cells. So far, no study has evaluated TLR9 protein expression on T cells and the consequenc -

\textbf{MATERIALS AND METHODS}

\textbf{Patients and general procedures}

Patients with NAFLD come from different centers of Buenos Aires whereas samples processing was entirely performed at the Clinical Hospital "José de San Martín". NAFLD patients group 1 comprised 68 individuals with biopsy-proven diagnosis of SS (n=34) or NASH (n=34). Its diagnosis was based on a daily alcohol consumption <20 g (females) or 30 g (males), absence of other causes of liver disease and abdominal ultrasound.\textsuperscript{17} The histological diagnosis was based on the NAFLD Activity Score\textsuperscript{18} and fibrosis staging\textsuperscript{18} with score values \(\geq 5\) as the cut-off for NASH. Liver biopsies and concomitant blood samples were used to evaluate TLR9 protein expression. NAFLD patients from group 2 were eight individuals who met the general criteria for NAFLD diagnosis but lacked histological diagnosis, hereafter called "NAFLD patients", used to evaluate TLR9 mRNA expression. Control group 1 included 11 metabolically healthy individuals who underwent anti-reflux surgery or cholecystectomy and did not have fatty liver which was confirmed by biopsy. Their biopsy samples were used to evaluate TLR9 protein expression. Control group 2 included 40 individuals recruited among the staff members of the Institute of Immunology, Genetics and Metabolism. All of the control individuals were given information of the study and those who intended to participate gave their written informed consent. Patients in the control groups did not received any medications within 6 months before the study, had low daily alcohol consumption, a body mass index (BMI) <25 kg/m\(^2\) and a waist circumference <80 cm (females) or 94 cm (males). Their blood samples were used in different experiments as indicated. The BMI and waist circumference were evaluated according to the World Health Organization crite-

\textbf{Reagents}

We used Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden), TRIZOL\textsuperscript{\textregistered} reagent and Roswell Park Memorial Institute 1640 medium (Life Technologies, Gaithersburg, MD, USA) and gentamicin, glutamine, ionomycin, fetal bovine serum, phorbol 12-myristate 13-acetate and brefeldin A (Sigma Chemical Co, Saint Louis, MO, USA). Phorbol 12-myristate 13-acetate was prepared in dimethyl sulfoxide at 25 \(\mu\)g/mL. We used BD™ IMag anti-human CD3 magnetic particles, PermWash™, Cytofix/Cytoperm, anti-human CD3 monoclonal antibody (UCHT1) for cell stimulation, anti-TLR9-phycocerythrin (PE) (eB72-1665) and anti-CD4-Peripherin-Chlorophyll-protein (PerCP) (SK3) from Becton-Dickinson (San Diego, CA, USA), anti-CD8-Fluorescein isothiocyanate (FITC) (UCHT-4), anti-CD69-PE (FN50) and anti-IFN-\(\gamma\)-PE (B27) from ImmunoTools GmbH (Friesoythe, Germany). The synthetic cytokine-phosphate-guanosine oligodeoxynucleotide (CpG-ODN) 2395 5’-tgcgtgtttrcg-ggccgcgcgcg-3’ (Thermo Fisher Scientific, Waltham, MA, USA) used as TLR9 agonist was solubilized in sterile endotoxin-free wa-

\textbf{Cell isolation}

Peripheral blood mononuclear cells were obtained using Ficoll-Hypaque gradients and resuspended in Roswell Park Memorial Institute 1640 medium 10% fetal bovine serum, 2 mmol/L L-glutamine, and 50 \(\mu\)g/mL gentamicin. Twice-enriched fractions of CD3\textsuperscript{+} T cells were isolated from peripheral blood mononuclear cells (PBMC) by negative selection using magnetic particles. Recovered cells were at least 98% CD3\textsuperscript{+}. We handled liver tissues at 4°C us-
ing a hands-on homogenization method, washed liver cell suspensions twice in phosphate buffered saline and harvested cells by centrifugation.

Quantitative polymerase chain reaction

Total RNA was isolated from CD3+ cells using TRIZOL® reagent and reverse transcribed using oligo (dT) primers and SuperScript™ II First-Strand Synthesis System (Invitrogen, Life Technologies, Carlsbad, CA, USA). Forward and reverse primers: TLR9, 5’-GAAGGGACCTCGAGTGTGAAG-3’ and 5’-GTAGGAAAGCAGG-3’; glyceraldehyde 3-phosphate dehydrogenase, 5’-CCACCACCTTGTCAAGCTCA-3’ and 5’-ACATGGCCCT-3’. Quantitative polymerase chain reaction was performed on a Stratagene Mx3005p RT-PCR Detection System (Agilent Technologies, La Jolla, CA, USA) using SYBR® Green PCR Master Mix (applied Biosystems, Life Technologies). Relative gene expression and fold change expression were calculated using the comparative Ct method.10

Intracytoplasmic TLR9 protein measurement

Suspension of 5×10⁵ isolated peripheral blood mononuclear cells or liver cells was centrifuged at 2,000 rpm for 5 minutes and incubated with anti-CD4-PerCP and anti-CD8-FITC in darkness at 4°C for 20 minutes. Fixed and permeabilized cells were incubated with anti-TLR9-PE or the isotype control in darkness at 4°C for 40 minutes, washed twice in PermWash™, resuspended in phosphate buffered saline 1X and assessed using flow cytometry. At least 100,000 events were acquired on a FACSCalibur flow cytometer (BD). The acquired data were analyzed using FlowJo software version 5.7.2 (Treestar, San Carlos, CA, USA). To analyze TLR9 expression, lymphocytes were gated on a forward and side-scatter plot and further gated based on CD4 or CD8 expression. TLR9 expression was expressed as a percent difference compared with isotype control using mean fluorescence intensity (MFI) values as follows: [(MFI of TLR9 / MFI of isotype control) - 1 / MFI of isotype control × 100].9

CD69 measurement

1×10⁶ isolated CD3+ cells were activated with soluble anti-CD3 (250 ng/mL) alone or in combination with 2 µM CpG-ODN in 24-well culture plates at 37°C for 24 hours. The cells were harvested by centrifugation, incubated with anti-CD4-PerCP, anti-CD8-FITC and anti-CD69-PE monoclonal antibodies in darkness at 4°C, and acquired data were analyzed using FlowJo software version 5.7.2 (Treestar, San Carlos, CA, USA). To analyze CD69 expression, lymphocytes were gated on a side-scatter plot and further gated based on CD4 or CD8 expression. CD69 expression was expressed as a percent difference compared with isotype control using mean fluorescence intensity (MFI) values as follows: [(MFI of CD69 / MFI of isotype control) - 1 / MFI of isotype control × 100].10

Table 1. Demographic and baseline data of the study cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>SS (n=34)</td>
<td>NASH (n=34)</td>
<td>SS vs. NASH</td>
<td>NAFLD (n=8)</td>
<td></td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>17/17</td>
<td>15/19</td>
<td>5/3</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 (33–59)</td>
<td>55 (41–58)</td>
<td>NS</td>
<td>39 (25–51)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>33.25 (26.50–39.38)</td>
<td>31.00 (28.80–40.00)</td>
<td>NS</td>
<td>31.23 (28.96–42.60)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.00 (99.00–116.30)</td>
<td>98.00 (94.00–105.00)</td>
<td>NS*</td>
<td>105.00 (96.00–120.00)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.50 (3.10–7.85)</td>
<td>4.95 (3.10–7.85)</td>
<td>NS</td>
<td>5.80 (2.40–8.10)</td>
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<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>102.00 (96.50–116.80)</td>
<td>127.00 (98.00–137.00)</td>
<td>NS</td>
<td>97.00 (88.00–108.00)</td>
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<tr>
<td>Insulin (µU/mL)</td>
<td>16.00 (10.60–30.50)</td>
<td>21.00 (9.12–26.50)</td>
<td>NS</td>
<td>20.80 (10.60–30.50)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>190.00 (168.00–233.00)</td>
<td>208.00 (192.00–247.00)</td>
<td>NS</td>
<td>185.00 (163.00–224.00)</td>
</tr>
<tr>
<td>Total triglycerides (mg/dL)</td>
<td>153.00 (88.50–218.50)</td>
<td>165.00 (98.00–189.00)</td>
<td>NS</td>
<td>153.00 (90.00–196.00)</td>
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<tr>
<td>AST (UI/L)</td>
<td>36.00 (27.50–43.50)</td>
<td>96.50 (73.50–121.25)</td>
<td>0.0003</td>
<td>75.00 (28.00–121.00)</td>
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<tr>
<td>ALT (UI/L)</td>
<td>47.50 (37.75–58.25)</td>
<td>137.00 (71.50–199.50)</td>
<td>0.0028</td>
<td>77.50 (41.25–165.80)</td>
</tr>
</tbody>
</table>

The table shows demographic and baseline data of NAFLD patients with biopsy-proven diagnosis of SS and NASH patients from group 1, and patients which met the general criteria for NAFLD but lacked the histological diagnosis from group 2. Results are summarized as median values (first-third quartiles). The Mann-Whitney U test was used to compare continuous variables between SS and NASH patients. Reference values: BMI: 25.00–29.90 kg/m² (overweight), greater than 30.00 kg/m² (obesity). Waist circumference: <84 cm (F) or 94 cm (M). HOMA-IR <2.5. Insulin: 2-20 µU/mL. Fasting blood glucose: <100 mg/dL. Total cholesterol: 150 to 199 mg/dL. Total triglycerides: <150 mg/dL. Aspartate transaminases: <48 UI/L. Alanine transaminases: <32 UI/L. SS, simple steatosis; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver diseases; M, male; F, female; BMI, body mass index; NS, no significant differences; HOMA-IR, homeostatic assessment model for insulin resistance; AST, aspartate transaminases; ALT, alanine transaminases.

*Comparisons were performed separately, between males or females with SS and NASH.
washed twice and resuspended in phosphate buffered saline 1X. A frequency index for CD69$^+$ cells was calculated within CD4$^+$ and CD8$^+$ cell compartments as the ratio of frequencies observed after treatments with anti-CD3+CpG-ODN and anti-CD3 alone.

**Intracytoplasmic IFN-γ staining**

To evaluate the frequency of IFN-γ$^+$ cells at baseline, peripheral blood mononuclear cells were stimulated with 25 ng/mL phorbol 12-myristate 13-acetate and 1 mM Ionomycin in the presence of 1 mM Brefeldin A at 37°C for 4 hours. After stimulation, cell sur-

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**Figure 1.** Intrahepatic and peripheral TLR9 protein expression. Lymphocytes were gated on a forward and side-scatter plot, and the lymphoid region was further gated based on CD4 or CD8 expression (not shown). (A, B) Intrahepatic TLR9 expression. (A) Representative flow cytometric histograms showing TLR9 expression within a CD4$^+$ cell compartment in a control subject, a patient with simple steatosis (SS), and a patient with nonalcoholic steatohepatitis (NASH). The ordinates and abscissas represent counts and fluorescence intensity, respectively. (B) The bar graph shows TLR9 expression in control subjects (n=11, group 1), patients with SS (n=13, group 1), and patients with NASH (n=14, group 1). TLR9 expression was expressed as a percentage difference in mean fluorescence intensity (MFI) value compared with the isotype control as follows: [(MFI of TLR9 / MFI of isotype control - 1 / MFI of isotype control) × 100]. We used the Kruskal-Wallis with Dunn’s multiple comparison test to compare data among the three groups. (C, D) Peripheral TLR9 expression. (C) Representative flow cytometric histograms showing TLR9 expression within intrahepatic CD4$^+$ and CD8$^+$ cell compartments in a control subject, a patient with SS, and a patient with NASH. (D) The bar graph shows TLR9 expression in control subjects and patients mentioned in (B). TLR9 expression was calculated as above. We used the Kruskal-Wallis with Dunn’s multiple comparison test to compare data among the three groups. In (B) and (D), the lines in each box represent the median values, and the horizontal boundaries of the boxes represent the first and third quartiles. The vertical error bars show the minimum and maximum values. *P<0.05; †P=0.0219; ‡P=0.0022 vs. controls.
face staining using anti-CD4-PerCP and anti-CD8-FITC monoclonal antibodies was performed as described above, and intracytoplasmic detection of IFN-γ was done as described for TLR9. Lymphocytes were gated on a forward versus size scatter plot, the lymphoid region was further gated based on CD4 or CD8 expression, and IFN-γ expression was measured in the selected populations. To evaluate the frequency of IFN-γ+ cells after co-stimulation, isolated PBMC were divided into 5×10⁵ cells per well each containing 500 µL of Roswell Park Memorial Institute medium and stimulated at 37°C for 18 hours in the presence of 1 mM Brefeldin A with 250 ng/mL soluble anti-CD3 alone or anti-CD3+2 uM CpG-ODN in 24-well culture plates. A frequency index for IFN-γ+ cells was calculated as described for CD69.

Statistical analysis

We used GraphPad Prism software (GraphPad, San Diego, CA, USA). We performed the two-tailed Mann-Whitney U test (two groups), the Kruskal-Wallis with Dunn’s multiple comparison test

Table 2. Relationships between TLR9 expression and necroinflammatory activity and fibrosis in NAFLD and comparison of TLR9 protein expression on intrahepatic T cells

<table>
<thead>
<tr>
<th>Cell type</th>
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<th>Score ≥2</th>
<th>P-value</th>
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<td>A. Comparison between patients grouped according to their histological scores of steatosis degree*</td>
<td>14</td>
<td>13</td>
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<tr>
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<tr>
<td>CD8+</td>
<td>179.30 (137.90–284.00)</td>
<td>192.9 (149.5–236.6)</td>
<td>NS</td>
</tr>
<tr>
<td>B. Comparison between patients grouped according to their histological scores of ballooning degeneration*</td>
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<td>13</td>
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</tr>
<tr>
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<td>165.4 (162.6–248.1)</td>
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<tr>
<td>CD8+</td>
<td>159.5 (138.3–336.3)</td>
<td>207.1 (178.8–226.8)</td>
<td>NS</td>
</tr>
<tr>
<td>C. Comparison between patients grouped according to their histological scores of lobular inflammation*</td>
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<td>0.0227</td>
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<tr>
<td>CD8+</td>
<td>179.3 (139.7–284.0)</td>
<td>207.1 (178.8–226.8)</td>
<td>NS</td>
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<tr>
<td>D. Comparison between patients grouped according to their fibrosis stage*</td>
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<td>159.5 (138.3–336.3)</td>
<td>207.1 (178.8–246.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

The table shows the relationship between TLR9 expression on intrahepatic T cells and necroinflammatory activity (A-C) or fibrosis (D). NAFLD patients included in Fig. 1 (n=27) were classified within two categories (as indicated in A-D), according to their histological scores of steatosis degree (A), ballooning degeneration (B), lobular inflammation (C), and fibrosis stage (D). Intrahepatic TLR9 protein expression was calculated as a percent difference compared with isotype control using MFI values as follows: [(MFI of TLR9 / MFI of isotype control - 1 / MFI of isotype control × 100]. We used the Mann-Whitney test to compare TLR9 expression between two categories, P<0.05 was considered statistically significant.

NS, no significant differences; NAFLD, nonalcoholic fatty liver diseases; MFI, mean fluorescence intensity.

*Mean fluorescence intensity median values (first quartile-third quartile) are included in the table.

Table 3. TLR9 expression on circulating CD4+ and CD8+ cells and its relationship with biochemical and clinicopathological variables of NAFLD

<table>
<thead>
<tr>
<th>Cell type</th>
<th>CD4+</th>
<th>CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI*</td>
<td>0.6636 (0.0260)</td>
<td>0.8833 (0.0016)</td>
</tr>
<tr>
<td>Waist circumference*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting blood glucose*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total triglycerides*</td>
<td>NS</td>
<td>0.9500 (&lt;0.0001)</td>
</tr>
<tr>
<td>AST*</td>
<td>0.7000 (0.0037)</td>
<td>0.6747 (0.0081)</td>
</tr>
<tr>
<td>ALT*</td>
<td>0.6929 (0.0042)</td>
<td>0.7011 (0.0052)</td>
</tr>
</tbody>
</table>

We used the Spearman’s rank correlation coefficients (r) to test the correlation between TLR9 expression and the anthropometric variables (BMI and waist circumference) or the plasma levels of selected metabolic variables, within peripheral CD4+ and CD8+ cell compartments. AST and ALT data were normalized according to the individual laboratory’s reference values.

BMI, body mass index; NS, no significant differences; AST, aspartate aminotransferases; ALT, alanine aminotransferases.

*Results summarized as r (P-value).
(three or more groups), and Wilcoxon paired test (intragroup comparisons). Data were summarized using the median, first and third interquartiles and minimum and maximum values. We used the Spearman’s rank correlation analysis to measure the statistical relations between TLR9 expression and continuous variables. P<0.05 was accepted as a significant value.

RESULTS

Characteristics of patients with NAFLD and control subjects

Anthropometric measurements, blood analysis and ultrasonography examination were performed at the beginning of the study (Table 1). All patients showed overweight or obesity and altered values of waist circumference and homeostasis assessment model for insulin resistance. We observed higher levels of aspartate and alanine aminotransferases in NASH than in SS patients from group 1. Anthropometric and biochemical values from control subjects fell into the reference ranges indicated (not shown). We described a direct relationship between plasma triglycerides concentration and fat content in NAFLD patients after comparing triglyceride levels between patients with steatosis scores “≤1” and “≥2” (P=0.0342, not shown).

Relationship between TLR9 expression, histological and clinicopathological features of NAFLD

We assessed TLR9 protein expression in liver cell suspensions (Fig. 1A, B). TLR9 expression was lowest within the CD4+ cell compartment in SS patients (P<0.05, vs. controls and vs. NASH patients) (Fig. 1B). TLR9 expression on CD8+ T cells was similar among groups. To get further insight into the biological relevance of these findings, we evaluated the relationship between TLR9 expression and NAFLD histological features (Table 2) after

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**Figure 2.** Synergistic interaction between T cell receptor and TLR9-mediated signaling in circulating cells. Isolated CD3+ cells from control subjects (n=6, group 2), patients with simple steatosis (SS) (n=6, group 1), and patients with nonalcoholic steatohepatitis (NASH) (n=6, group 1) were cultured in Roswell Park Memorial Institute 1640 medium, stimulated with anti-CD3 alone or with anti-CD3+CpG-ODN. After 24 hours of culture, surface staining of CD4, CD8, and CD69 antigens was performed. (A) Representative dot plots showing cells gated in the CD8+ region in a control subject, a patient with SS, and a patient with NASH. Dot plots show the percentage of CD69+ cells observed after treatment. (B) The frequency index for CD69+ cells within CD4+ and CD8+ cell compartments was calculated as a ratio of frequencies observed after treatment with anti-CD3+CpG-ODN or anti-CD3 alone. The line in each box represents the median, the horizontal boundaries of the boxes represent the first and third quartiles, and the vertical error bars show the minimum and maximum values. We used the Kruskal-Wallis with Dunn’s multiple comparison test to compare data among the three groups. *P<0.005; †P<0.0005.
Figure 3. Synergistic interaction between TLR9 and T cell receptor-mediated signaling for differentiation of CD8+ cells. Peripheral blood mononuclear cells from control subjects were cultured in Roswell Park Memorial Institute 1640 medium and stimulated with soluble anti-CD3 alone or with anti-CD3+CpG-ODN for 18 hours in the presence of Brefeldin A. After treatment, surface (CD4 or CD8) and intracellular (IFN-γ) staining was performed. Lymphocytes gated on a forward side and side scatter plot were further gated based on CD4 or CD8 expression (not shown). (A) Representative dot plots showing the frequency of IFN-γ+ lymphoid cells observed within the CD8+ gate after treatment of PBMC from a control subject, a simple steatosis (SS) patient, and a nonalcoholic steatohepatitis (NASH) patient with anti-CD3 and anti-CD3+CpG-ODN. (B) Experiments performed with control subjects (n=5, group 2), patients with SS (n=5, group 1), and patients with NASH (n=5, group 1). The frequency index for IFN-γ+ lymphoid cells was calculated for each cell compartment as a ratio of frequencies observed after treatment with anti-CD3+CpG-ODN or anti-CD3 alone. The line in each box represents the median, the horizontal boundaries of the boxes represent the first and third quartiles, and the vertical error bars show the minimum and maximum values. We used the Kruskal-Wallis with Dunn’s multiple comparison test to compare data among the three groups. *P=0.0400, †P=0.0047.

Figure 4. Frequency of IFN-γ+ circulating cells in control subjects and patients with NAFLD. Peripheral blood mononuclear cells from control individuals (n=11, group 2), patients with simple steatosis (SS) (n=8, group #1), and patients with nonalcoholic steatohepatitis (NASH) (n=9, group 1) were stimulated for 4 hours with the protein kinase C stimulant phorbol 12-myristate 13-acetate and ionomycin in the presence of Brefeldin A before surface (CD4 or CD8) and intracellular (IFN-γ) staining. To analyze the frequency of IFN-γ+ cells, lymphocytes gated on a forward side and side scatter plot were further gated based on CD4 or CD8 expression (not shown). The bar graph shows the percentage of IFN-γ-producing CD4+ and CD8+ cells. The line in each box represents the median, and the horizontal boundaries of the boxes represent the first and third quartiles. The vertical error bars show the minimum and maximum values, *P<0.01, †P=0.0005. We used the Kruskal-Wallis test to compare data among the three groups.
stratification of NAFLD patients according to their histological scores within the categories indicated in Table 2. Our results showed a positive association between TLR9 expression in CD4+ cells and liver damage. Then, we assessed TLR9 protein expression in peripheral blood mononuclear cells from the individuals previously evaluated (Fig. 1C, D). SS patients showed the lowest TLR9 expression within CD4+ (P=0.0219) and CD8+ (P=0.0022) cell compartments (Fig. 1D). A quantitative polymerase chain reaction-based analysis of TLR9 mRNA in CD3+ T cells confirmed a decreased expression of TLR9 mRNA in about 50% of patients (not shown). To assess the relationship between TLR9 expression at peripheral blood and clinic-pathological variables relevant to NAFLD, we performed a correlation analysis (Table 3). NAFLD patients with a lower alteration of BMI and aminotransferases activity exhibited a lower TLR9 expression within both cell compartments. We also found a positive association between plasma triglycerides concentration and TLR9 expression in peripheral CD8+ cells.

Synergistic interaction between signals coming from T cell receptor and TLR9 in circulating T cells

Given that a dysfunctionality of Kupffer cells in NASH might enhance the influx of TLR9 ligands into the systemic circulation and interact with T cells, we explored the consequences of TLR9-mediated signaling of circulating T cells. Because cell activation is a prerequisite for T cell differentiation, we assessed CD69 upregulation and IFN-γ production as phenotypic markers of these processes. We employed isolated CD3+ cells for the assessment of cell activation to avoid the indirect effects of non-T cells, and the higher affinity of non-T cells for TLR9 ligands (Fig. 2). Regarding CD69 expression, the simultaneous addition of anti-CD3 and CpG-ODN only provided co-stimulation to CD8+ cells (intragroup comparisons: anti-CD3+CpG-ODN vs. anti-CD3 alone, not shown). Upregulation of CD69 was lowest in SS patients (P<0.01 vs. controls, P<0.05 vs. NASH patients). As for CD69, the addition of CpG-ODN did not alter intracellular IFN-γ production. Thus, our results demonstrated that T cells co-stimulation promoted the lowest activation and differentiation of circulating CD8+ cells in SS patients. The small size of our liver biopsies precluded its use for functional studies.

Frequency of IFN-γ+ cells at baseline

We measured the frequency of circulating IFN-γ+ T cells (Fig. 4). The frequency of IFN-γ+ cells within CD4+ (P<0.01) and CD8+ (P=0.0005) cell compartments was higher in NASH patients than control subjects. SS patients and control subjects showed similar frequencies of type 1 CD8+ cells and T helper 1 cells, respectively.

DISCUSSION

We found positive associations between TLR9 expression on intrahepatic CD4+ T cells, necroinflammation and liver fibrosis, and between TLR9 expression on peripheral CD4+ and CD8+ T cells and clinic-pathological alterations of NAFLD such as BMI, plasma triglycerides concentration and aminotransferases activity. Relevant alterations of NAFLD in this study reflect liver disease or damage or predict the steatosis degree.

The regulation of the inflammatory response in peripheral blood mononuclear cells is dependent on the metabolic status. Since metabolic alterations associated with NAFLD are similar among our patients, the decreased expression of TLR9 on T cells from SS patients may be independent from the metabolic context, particularly from insulin even though it down modulates the expression of TLRs on T cells.

We showed an overall downregulation of TLR9 on T cells from SS patients affecting intrahepatic CD4+ T cells as well as peripheral CD4+ and CD8+ T cells. It can be interpreted that this feature was likely a protection adaptation from hepatocellular injury whereas the observed unvarying expression in patients with NASH may be a failure of this regulatory mechanism. The cytoplasmic tails of all TLRs contain a toll-interleukin 1 receptor (TIR) domain. The TIR domain of TLR9 provides a binding site for an ubiquitin-protein ligase regulating its abundance and activity. Since ubiquitination of proteins mediates liver protection of patients with NAFLD, an increased ubiquitination of TLR9 might be involved in TLR9 downregulation on T cells from SS patients.

Our study confirms previous data concerning a synergism be-
between T cell receptors and TLR9 during the induction of IFN-γ. Furthermore, it reveals a milder response in CD8+ cells from SS patients following its co-stimulation. Precise mechanistic insight exists to priming effects of IFN-γ on macrophage activation towards an inflammatory phenotype. We can speculate that if the consequences of the co-stimulation were similar for intrahepatic and peripheral T cells, the decreased intrahepatic TLR9 expression observed in SS patients would promote a low number of type 1 CD8+ cells, favor the differentiation of anti-inflammatory Kupffer cells and protect against liver injury. In NASH, a failure of this regulatory mechanism would allow the progression of liver injury.

As compared with controls, NASH patients showed similar TLR9 expression and similar responses after TLR9 ligation of T cells. However, NASH patients showed a higher frequency of IFN-γ-producing cells than SS patients and controls at baseline. In the context of NASH, the presence of strong pro-inflammatory adipokines and cytokines promoting type 1 CD8+ cells differentiation supports these findings. The enrollment of patients in several centers from the specific area of Buenos Aires enhanced the possibility of generalizing our results to similar patients in similar settings. However, a limitation of our study was that a biopsy-proven diagnostic of NAFLD was unavailable from one of the participating centers. As we did not have an appropriate pilot study to estimate the sample size, we used information from previous studies in the same population, which allowed us to identify differences between groups provided that those differences genuinely existed in the population.

A decreased expression of TLR9 and a limited production of IFN-γ by T cells from SS patients could play a protective role. T cells from NASH patients lack this mechanism and, furthermore, maybe influenced by strong pro-inflammatory signals of the microenvironment supporting the observed overproduction of IFN-γ. The impact of TLR9-mediated triggering of T cells within regulatory circuits potentially involved in NAFLD deserves further investigation.

Authors’ contribution

NSA and ACCH designed the study; NSA, CCG and LAB performed the experiments and collected data; BA, DP and JB selected the patients, provided the samples and supervised clinical aspects; ACCH, LAB and NSA analyzed and interpreted the data; ACCH wrote the draft of the article; LC, NSA and ACCH made a critical revision and edited the article, acquired financial support, and made the final approval of the version to be published.

Acknowledgments

This research was supported by the University of Buenos Aires (grant numbers 100008BA and 00001BA) and the National Council for Scientific and Technological Investigation (grant number 0051).

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES


Dear Editor,

Transjugular intrahepatic portosystemic shunt (TIPS) is an established treatment for refractory ascites as well as variceal bleeding. Unfortunately, shunt dysfunction (stenosis/occlusion) commonly occurs in 13% to 50% of TIPS patients, depending on the stent type (covered or bare metal) placed. We report a case of occluded TIPS with splenic and superior mesenteric veins (SMV) thrombosis, which was managed with mechanical and suction thrombectomy, following angioplasty and stenting.

A 64-year-old hepatic cryptogenic cirrhosis male patient, complicated by portal hypertension and refractory ascites was treated in 2011 by placement of Viatorr TIPS Endoprosthesis (W.L. Gore & Associates, Newark, DE, USA; graft length lined 7 cm and unlined 2 cm, internal diameter 10 mm). On March 2018 he was referred to the authors’ hospital because of progressive increase of weight and abdominal circumference associated to dyspnea and lower limbs edema. The patient was in Child-Pugh B8 class, with normal white blood cell (5.23×10⁹/L) and platelet count (155×10⁹/L).

The annual routine Doppler ultrasound (US) performed three months before showed an evidently expanded stent between the right portal and right hepatic vein, with regular peak shunt velocity. The main portal vein flow velocity and directionality in the intrahepatic portal veins were normal. Abdominal US at the authors’ hospital, revealed an absent flow within the shunt, more precisely at the mid-shunt, the portal and hepatic venous end. Additionally, large ascites with marked abdominal distension was evident. Abdominal computed tomography angiography confirmed a subacute thrombosis of the main portal trunk, which extended to the right portal vein, and spleno-mesenteric confluence (Fig. 1).

The occluded TIPS was navigated using 5 Fr catheter and guidewire via transjugular approach, and venography confirmed complete occlusion of TIPS and thrombosis in main portal vein (Fig. 2). Initially, mechanical thrombectomy was performed using 10 Fr Aspirex S system (Straub Medical AG, Wangs, Switzerland) (Fig. 3A). A partial outflow within the shunt with residual thrombosis persistence in spleno-mesenteric confluence and decreased caliber was demonstrated with portal venogram images after the thrombectomy and aspiration procedure. Therefore an 8 mm and 10 mm balloon angioplasty was performed in the shunt and spleno-mesenteric confluence up to the main portal vein. A post balloon angioplasty venography still revealed an unsatisfying flow. Therefore four E-Luminexx 12×40 mm stents (Bard GmbH/Angiomed, Karlsruhe, Germany) were placed: two from the spleno-
mesenteric confluence up to the main portal vein and additional two within the shunt. The portal venogram images demonstrated a rapid contrast medium outflow toward the shunt and the hepatic veins (Fig. 3B). Anticoagulant treatment with fondaparinux sodium, 5 mg subcutaneously once daily was administered for 30 days.

An US evaluation, performed 1 month after TIPS revision, showed stent patency between the right portal and hepatic vein, in the distal tract of the splenic vein, which extended to the spleno-mesenteric confluence with a regular peak shunt velocity. A minimal thrombosis with a regular blood flow direction and velocity of the spleno-mesenteric confluence, the distal tract of the main portal vein extended to the bifurcation and to the right branch of the portal trunk was revealed. Seven months after the intervention, US and CT scan demonstrated a complete thrombosis resolution of the shunt, intrahepatic portal vein branches and the main portal trunk up to the spleno-mesenteric confluence (Fig. 4).

Technical variables during TIPS creation, thrombophilic risk factors, hypercoagulable state and/or large competitive spontaneous splenorenal shunts, determining flow reduction in TIPS, can possibly cause stent occlusion. Furthermore, Yue-Meng et al. demonstrated that low white blood cell count, high Child Pugh class and severe ascites are independent predictors of portal vein thrombosis in TIPS-treated patients with cirrhosis. An early detection and correction of TIPS failure is crucial. The most commonly applied technique for TIPS occlusion is recanalization performed through the jugular vein with a hydrophilic guide wire or a Cook needle (Colapinto or Rosch-Uchida), although the access to the shunt is sometimes difficult. Therefore, alternative therapeutic strategies could be: 1) fluoroscopically guided transhepatic stent puncture, 2) creation of a new shunt via direct cavoportal puncture or with the so-called ‘gun-sight approach’ or 3) creation of a new parallel shunt.

Mechanical thrombectomy (MT) or pharmacologic thrombolysis, directly through the shunt, can manage thrombotic occlusion and stenosis of TIPS and of the portal venous system. Balloon and suction embolectomy, basket extraction of clots and other mechanical thrombectomy with special devices are reported MT techniques which may be used to reopen the shunt and the thrombosed venous system. These MT techniques can be performed solely or in combination. Obvious advantages of MTs could be the rapid removal of the thrombus without the need for prolonged lytic infusions, which is known for potential life threatening bleeding complications. Hilliard et al. recently described
the use of US-assisted thrombolysis for TIPS occlusion, demonstrating decreased risks associated with thrombolytic agents shortening treatment times. However, this technique requires additional equipment required compared to the standard catheter-direct thrombolysis and is therefore more expensive. In our case an subacute TIPS thrombosis extended into the portal, splenic and SMV, occurred in a cirrhotic decompensated patient (Child Pugh class B and with grade III ascites), which has been treated with the jugular vein approach and thrombectomy in combination with three mechanical techniques: mechanical and suction thrombec-
tomy, following angioplasty plus stenting. This approach may be useful approach to treat TIPS occlusion where single conventional techniques might be ineffective or where thrombolytic therapy is contraindicated.

**Authors’ contributions**

Study concept and design: NDM, CDS, LM. Data acquisition: NDM, FP. Data analysis and interpretation: NDM, CDS, LM. Drafting of the manuscript; critical revision of the manuscript for important intellectual content: NDM, CDS, LM. Study supervision:

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**Figure 3.** (A) Venography after mechanical and suction thrombectomy shows partial recanalization of TIPS tract and main portal vein with contrast media filling in the collateral veins. (B) Venography after angioplasty plus stenting demonstrates a rapid contrast medium outflow toward the shunt and the hepatic veins without visualization of the collateral veins. TIPS, transjugular intrahepatic portosystemic shunt.

**Figure 4.** A computed tomography scan performed 6 months after the procedure showing the complete resolution of the thrombosis of the shunt, the intrahepatic portal vein branches and the main portal trunk up to the spleno-mesenteric confluence.
RM, GR, LM. All authors approved the final version of the manuscript.

Acknowledgements
The authors are indebted to Mrs. Franziska Michaela Lohmeyer for language editing, and proofreading.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Dear Editor,

We recently had the opportunity to read the brilliant paper by Thakral and Simonetto "Hyperammonemic encephalopathy: An unusual presentation of fibrolamellar hepatocellular carcinoma" in the Clinical and Molecular Hepatology. We would appreciate to share some insights about this interesting theme.

Fibrolamellar hepatocellular carcinoma (FLHCC) is indeed a rare primary hepatic tumor that arises in non-cirrhotic livers, more common in males (male to female ratio 1.7), with age-specific incidence with two peaks between ages of 10–30 and 60–69 (although being a tumor usually believed to affect mostly very young individuals) and is associated with better 5-year survival than conventional hepatocellular carcinoma (HCC). Although FLHCC was usually referred as a subtype of HCC, recent researches have demonstrated that FLHCC is actually an independent entity, with distinctive molecular tumor profile, histological features and clinical presentation.

One of the most feared complications associated with FLHCC is the development of acute onset hyperammonemic encephalopathy (HAE), which was first reported by Sethi et al. in 2009, this condition is associated with high mortality. High mortality was related to the ignorance of the physiopathology of HAE in patients with FLHCC. Some authors have proposed some explanations, such as portosystemic shunt. In 2017, we published a new proposal of the physiopathology of this complication of FLHCC, and initiated with the very unique mutation (a heterozygous deletion of chromosome 19) that is responsible for the development of the tumor. This mutation is responsible for overexpression of a chimeric DNAJB1-PRKACA kinase and Aurora Kinase A that culminate with c-Myc and ornithine decarboxylase dysfunction and result in depletion of amino acids crucial to urea cycle function. This urea cycle dysfunction is then responsible for the accumulation of ammonia in the bloodstream and occurs HAE.

The importance of this finding was, once understanding that an urea cycle disorder due to metabolites consumption was responsible for the hyperammonemia, to guide the development of new medical treatment options based on a combination of ammonia scavenger drugs (sodium benzoate and phenylbutyrate) with amino acids supplementation (citrulline, ornithine and arginine), thus reducing the mortality of FLHCC-related HAE and allowing complete clinical recovery from HAE.

So, we think that question stated by Hashash et al. in 2012...
“What is the cause of acute hepatic encephalopathy in a young patient?” regarding an 18-year-old patient with a large FLHCC has been answered, including its precise pathophysiology. Nevertheless, a few more steps must still be taken. First, the molecular basis of the proposed pathophysiology is needed to be proven. Second, there were more studies are warranted to demonstrate the efficacy and safety of these new treatment options. Third, this treatment regimen for hyperammonia may be useful for other causes of liver-related HAE such as portosystemic shunt, acute liver failure and cirrhosis.

**Author’s contributions**

Rodrigo Cañada trofo Surjan designed and wrote the paper, designed the proposal of the pathophysiology described and performed final approval of the article. Elizabeth Santana dos Santos was involved in direct patient care and data collection. Sergio do Prado Silveira performed data collection and paper drafting. Fabio Ferrari Makdissi and Marcel Autran Cesar Machado performed critical review of the article.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

Dear Editor,

I have interestingly read the article entitled “The dilemma of differentiating between acute hepatitis B and chronic hepatitis B with acute exacerbation: Is quantitative serology the answer?” by Lall et al.1 It is difficult to distinguish between acute hepatitis B (AHB) and acute exacerbation of chronic hepatitis B (CHB-AE) as the two conditions have very similar clinical, biochemical, and serological findings. However, since the two conditions differ in their treatment strategy, prognosis, and impact on the public health, it is very important to distinguish them from one another. In this study, the authors suggested the value of 20.5 signal-to-cutoff (S/CO) of IgM anti-HBc can be used to differentiate between AHB and CHB-AE. However, before interpreting and applying these results in clinical practice, several issues should be kept in mind.

IgM anti-HBc has been traditionally considered as a specific marker of AHB, since its detection is observed at high levels during primary immune response after HBcAg interaction with immune cells.2 Recently, the introduction of assays with high sensitivity has made it possible to detect low levels of IgM anti-HBc in CHB-AE.3 Therefore, high levels of IgM anti-HBc may be suggestive of AHB, whereas low levels may suggest CHB-AE. However, currently available data on differentiating AHB from CHB-AE are not only limited, but they are also not robust and have variations in differentiating criteria or assays in each study. For example, Lall et al.1 defined the CHB-AE group as a case of HBsAg lasting for more than 6 months, while Kumar et al.2 defined it differently as a case of HBsAg lasting for at least 12 months, along with the development of clinical, biochemical, radiologic, or histologic evidence of chronic liver disease on follow-up. Ethnics, hepatitis B virus (HBV) genotype, and individualized immune response can also affect IgM anti-HBc levels, making it difficult to determine the standardized cut-off index for distinguishing between AHB and CHB-AE.

Most studies defined a group of AHB as patients who do not show progression to chronic liver disease. Since the degree of hepatic fibrosis by elastography or serological markers can be significantly influenced by liver damage, some authors proposed that fibrosis test should be postponed for at least 3 months after stabilization of alanine aminotransferase (ALT) or acute flare in order to restore its reliability.4,5 In this study, only baseline aspartate aminotransferase (AST)/ALT ratio and AST to platelet ratio index were analyzed; however, continuous changes in follow-up after stabilization could be more clinically meaningful.

Several studies have introduced the analysis of serologic markers, including IgM anti-HBc and their kinetics, as a helpful test for distinguishing between AHB and CHB-AE.1,2,6-11 Table 1 summarizes the proposed discriminant factors from published studies to date.

### A challenge in distinguishing between acute hepatitis B and acute exacerbation of chronic hepatitis B

Yang-Hyun Baek

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**Abbreviations:**

AFP, alphafetoprotein; AHB, acute hepatitis B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVT, anti-viral therapy; CHB-AE, acute exacerbation of chronic hepatitis B; HBV, hepatitis B virus; S/CO, signal-to-cutoff

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**Editor:** Seung Up Kim, Yonsei University College of Medicine, Korea

**Received:** Feb. 27, 2020 / **Revised:** Feb. 28, 2020 / **Accepted:** Mar. 5, 2020

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Avidity index of IgG anti-HBc suggests a useful factor in the differential diagnosis of AHB and CHB-AE. IgG avidity is defined as the strength with which IgG binds to antigenic epitopes expressed by a given protein, which increases as IgG matures. Rodella et al. reported that an anti-HBc avidity index <0.7 indicated the possibility for AHB. Although IgG avidity index may be helpful, its standardized technique and clinical use are still limited. Some studies suggested that serum HBV-DNA levels are lower in AHB than in CHB-AE, although no significant difference was demonstrated in this study. Lower levels of HBV-DNA in AHB indicated that they have been controlled by appropriate immune response from incubation period, while high levels of virus exist under insufficient immune control in CHB. However, viral replication could show various levels, and it is difficult to set a standardized cut-off level of serum HBV-DNA. It might be helpful to monitor DNA kinetics with viral replication for differential diagnosis.

Han et al. showed that elevated alpha-fetoprotein (AFP) were found in fewer patients with AHB than in those with CHB (26.1% vs. 63.2%). They hypothesized that hepatocyte necroinflammation can trigger elevation of AFP levels with fibrosis or cirrhosis already present.

In summary, high IgM anti-HBc titer, low IgG anti-HBc avidity index, low serum HBV-DNA, HBV-DNA kinetics of rapid decline, and low AFP level suggest the probability of AHB rather than CHB. However, there is still insufficient evidence to present a standardized cut-off value for differential diagnosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient</th>
<th>Differential factor</th>
<th>Cut-off index</th>
<th>Tendency</th>
<th>Method/assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kumar et al.</td>
<td>AHB (n=49)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>≥1:1,000 &lt;0.5 pg/mL</td>
<td>AHB AHB</td>
<td>MEIA Hybrid capture</td>
<td>77.6% 95.9%</td>
<td>70% 86.6%</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>AHB (n=20)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>&gt;2.4–2.5 S/CO ≥0.7</td>
<td>AHB CHB</td>
<td>AxsYM CORE-M CLIA</td>
<td>2.42 (90%) 2.46 (85%) 2.46 (90%)</td>
<td>98.9% 99%</td>
</tr>
<tr>
<td>Rodella et al.</td>
<td>AHB (n=36)</td>
<td>IgG avidity index</td>
<td>&gt;10 S/CO &gt;0.7</td>
<td>AHB CHB</td>
<td>CLIA AxsYM assay</td>
<td>100% 98.9%</td>
<td>99%</td>
</tr>
<tr>
<td>Han et al.</td>
<td>AHB (n=138)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>&gt;1:10,000 &lt;10^5 copies/mL elevated AFP</td>
<td>AHB AHB CHB-AE</td>
<td>EIA PCR method ECLIA</td>
<td>96.2% 98.9%</td>
<td>93.1% 99%</td>
</tr>
<tr>
<td>Dao et al.</td>
<td>AHB-ALF (n=60)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>&gt;5 S/CO</td>
<td>AHB-ALF</td>
<td>IgM capture immunoassay PCR method</td>
<td>86% 89%</td>
<td></td>
</tr>
<tr>
<td>Park et al.</td>
<td>AHB (n=53)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>≥8 S/CO &lt;5.5 log10 IU/mL</td>
<td>AHB AHB</td>
<td>CLIA PCR method</td>
<td>96.2% 81.1%</td>
<td>89.7% 72.4%</td>
</tr>
<tr>
<td>Thangarajan et al.</td>
<td>AHB (n=26)</td>
<td>IgM anti-HBc HBV-DNA</td>
<td>≥12.14 S/CO &gt;15,390 IU/mL</td>
<td>AHB AHB CHB-AE</td>
<td>ECLIA</td>
<td>76.9% 78.6%</td>
<td>71.4% 46.2%</td>
</tr>
<tr>
<td>Lall et al.</td>
<td>AHB (n=89)</td>
<td>IgM anti-HBc PT-INR</td>
<td>&gt;20.5 S/CO &lt;1.27</td>
<td>AHB AHB</td>
<td>CLIA</td>
<td>93.3% 92.7%</td>
<td></td>
</tr>
</tbody>
</table>

AHB, acute hepatitis B; CHB-AE, acute exacerbation of chronic hepatitis; HBV, hepatitis B virus; MEIA, microparticle enzyme immunoassay; CLIA, chemiluminescent immunoassay; AFP, alpha-fetoprotein; EIA, enzyme immunoassay; PCR, polymerase chain reaction; ECLIA, electro-chemiluminescence immunoassay; ALF, acute liver failure; PT-INR, prothrombin time-international normalized ratio.
sexual behavior, or intravenous drug use. However, his mother had CHB. His laboratory findings were as follows: AST, 1,265 U/L; ALT, 1,961 U/L; total bilirubin, 21.7 mg/dL; direct bilirubin, 13.9 mg/dL; prothrombin time, 12.2 seconds; and international normalized ratio, 1.06. He tested positive for HBsAg, IgM anti-HBc, and HBeAg with negativity for hepatitis A, hepatitis C, hepatitis D, and hepatitis E. IgM anti-HBc by chemiluminescent immunoassay was 6 S/CO, and HBV-DNA titer was 906,844 IU/mL. It was very difficult to differentiate AHB from CHB-AE. We decided to start anti-viral therapy (AVT) based on the history of his mother’s hepatitis B and low IgM anti-HBc titer with high DNA viral load. The patient’s symptoms and laboratory findings improved day by day; and since he had to leave for Vietnam again, he visited our hospital only twice after discharge. The patient’s HBV-DNA was negative 3 weeks after the AVT began, and HBeAg was negative on the first outpatient visit. HBsAg seroconversion was also achieved in his second visit; therefore, the HBsAg loss accompanied by a rapid decline of HBV-DNA suggested AHB rather than CHB-AE, despite low IgM anti-HBc and high viral load on admission.

In South Korea, the overall prevalence of HBsAg decreased to 3% after the introduction of the national immunization program for HBV and perinatal transmission prevention program. Despite the low prevalence rate, unvaccinated people may be vulnerable to AHB, and the vaccination rate for patients with AHB from 2012–2017 was actually less than 10%. Focusing on effective and implementable prevention strategy for HBV is more preemptive than distinguishing between AHB and CHB-AE.

Author’s contribution
Yang-hyun Baek contributed to analysis of data, concept of design and writing the manuscript.

Conflicts of Interest
The author has no conflicts to disclose.

REFERENCES

Dear Editor,

I recently read an interesting article by Alejandra et al.\(^1\) which showed the relationship between toll-like receptor 9 (TLR9), cluster of differentiation 69 (CD69) expression, and interferon-\(\gamma\) expression related to liver injury in patients with nonalcoholic fatty liver disease. Non-alcoholic fatty liver disease involves a comprehensive process from simple fatty liver to nonalcoholic steatohepatitis (NASH), which includes hepatocellular damage (hepatocyte ballooning) due to infiltration of inflammatory cells as well as fat accumulation, which thereby progresses to NASH-associated fibrosis and NASH-associated cirrhosis.\(^2,3\) The authors investigated the expressions of TLR9 and CD69, as well as the frequency of interferon-\(\gamma\) positive cells after anti-CD3 and TLR9 ligand (CpG oligodeoxynucleotid) stimulation, which were examined in peripheral blood cells isolated from normal control, simple steatosis (SS) patients, and NASH patients. In comparing the CD4 and CD8 T cells of SS patients with the cells of normal controls and NASH patients, a number of different aspects were found: 1) TLR9 expression on T cells, 2) CD69 expression by anti-CD3 and TLR9 ligand stimulation, and 3) interferon-\(\gamma\) positive cells. NASH patients had a significantly higher percentage of interferon-\(\gamma\) positive circulating cells compared to the others. In fact, the decreased expression of TLR9 on T cells and reduced interferon-\(\gamma\) expressing T cells in SS patients may play a protective role against liver damage. However, there are several things to consider regarding this hypothesis. I have briefly summarized the results, as shown in the table below, to enhance the readers’ understanding (Table 1).\(^1\)

First, the authors analyzed TLR9 expressions in both intrahepatic and peripheral CD4 T and CD8 T cells. However, only peripheral blood mononuclear cells were used to examine the expressions of CD69 or interferon-\(\gamma\). Since hepatocellular damage in NASH patients could actually be caused by intrahepatic immune cells, it remains somewhat questionable whether peripheral blood analysis can really reflect intrahepatic immune cells. Also, intrahepatic immune cells showed significant differences in phenotype and composition of immune cells located in other organs, such as the spleen lymph nodes, and etc.\(^5\)

Second, it is not clear whether these processes were caused by a change in TLR9 ligand responsiveness for T cell activation or by other factors, such as a difference in the influx of TLR9 ligands into the liver between SS and NASH patients. The authors suggested that in patients with SS, T cell activation and induction of interferon-\(\gamma\) positive cells were blunted due to low TLR9 stimulation on CD8 T cells, resulting in less liver damage compared to

Does limited expression of toll-like receptor 9 actually contribute to T cell activation and liver damage in non-alcoholic steatohepatitis?

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**Abbreviations:**
- CD3: cluster of differentiation 3
- CD4: cluster of differentiation 4
- CD8: cluster of differentiation 8
- CD69: cluster of differentiation 69
- NASH: nonalcoholic steatohepatitis
- SS: simple steatosis
- TLR9: toll-like receptor 9

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**Editor:** Seung Up Kim, Yonsei University College of Medicine, Korea

**Received:** Feb. 27, 2020 / **Revised:** Mar. 4, 2020 / **Accepted:** Mar. 5, 2020

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NASH patients. It seems that T cells of each group may appear to show a difference in activation by TLR9 ligand stimulation under controlled laboratory conditions. However, the results for comparing the actual interferon-γ positive circulating T cells isolated from patients did not show any significant differences in SS patients compared to NASH patients. This suggests that other factors could also take part in leading to liver damage under in vivo conditions. Indeed, SS progresses to NASH via a complex process involving many factors, such as genetic susceptibility, various environmental effects, and immunological factors. For example, when TLR9 ligand enters the liver, hepatic stellate cells and Kupffer cells may react with the ligand and produce pro-inflammatory cytokine, which induces secondary activation of CD4 and CD8 T cells. Therefore, it would be more clear if additional supporting results were available, such as a ratio comparison of intrahepatic interferon-γ positive CD4 and CD8 T cells in normal control, SS model, and NASH model isolated from mice lacking TLR9 in the myeloid cells (TLR9 f/f X Lysozyme M-Cre mouse). Objective evidence should be provided on whether there is actually a difference in TLR9 ligand influx into the liver between NASH and SS patients. Further experiments on the aforementioned points would help solve the questions raised in this study.

**Conflicts of Interest**

The author has no conflicts to disclose.

## REFERENCES


### Table 1. TLR9, CD69, and interferon-γ expression on T cell for control, SS, and NASH patients

<table>
<thead>
<tr>
<th></th>
<th>TLR ligand influx increase/decrease</th>
<th>TLR9 expression level</th>
<th>CD69 expression after anti-CD3, TLR9 ligand stimulation</th>
<th>Interferon-γ expression levels by anti-CD3 and TLR9 ligand stimulation</th>
<th>Interferon-γ expression level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Intrahepatic</td>
<td>–</td>
<td></td>
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<tr>
<td>Peripheral</td>
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<tr>
<td><strong>SS</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Intrahepatic</td>
<td>–</td>
<td>CD4 T ↓</td>
<td>CD4 T ↓</td>
<td>CD4 T ↓</td>
<td>CD4 T ↑</td>
</tr>
<tr>
<td>Peripheral</td>
<td>–</td>
<td>CD4 T ↓</td>
<td>CD4 T ↓</td>
<td>CD4 T ↓</td>
<td>CD4 T ↑</td>
</tr>
<tr>
<td><strong>NASH</strong></td>
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<tr>
<td>Intrahepatic</td>
<td>–</td>
<td>CD4 T →</td>
<td>CD4 T →</td>
<td>CD4 T →</td>
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<tr>
<td>Peripheral</td>
<td>–</td>
<td>CD4 T →</td>
<td>CD4 T →</td>
<td>CD4 T →</td>
<td>CD4 T ↑</td>
</tr>
</tbody>
</table>

TLR, toll-like receptor; CD, cluster of differentiation; SS, simple steatosis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; NASH, nonalcoholic steatohepatitis.
Letter to the Editor

Intricate interpretation of etiology-specific outcome comparison in patients with hepatocellular carcinoma

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Dear Editor,

We have read Kumar et al.’s study¹ published in the previous issue of Clinical and Molecular Hepatology with great interest, which compared the characteristics and outcomes in patients with nonalcoholic steatohepatitis (NASH)-associated hepatocellular carcinoma (HCC) versus those with alcoholic steatohepatitis (ASH)-associated HCC. The study reported several differences in characteristics, such as age, sex, and comorbidities, between the two groups, whereas their survival rates as well as liver- and tumor-related factors were comparable. Although the current study was a retrospective analysis of a single-center cohort, it still offers a number of relevant points for further consideration.

First, patients with nonalcoholic fatty liver disease (NAFLD) or alcohol-related liver disease (ALD) are prone to deficient surveillance.²,³ Patients with undiagnosed alcohol-related cirrhosis also contribute to the low rate of surveillance as well as late diagnosis of HCC, due to their low compliance with regular appointments.⁴ NASH-related cirrhosis is a well-known indication of surveillance for HCC. However, for patients with non-cirrhotic NASH (or even non-NASH NAFLD), there has been no consensus on whether to offer surveillance or not. In Kumar et al.’s study,¹ the frequencies of non-cirrhotic patients with NASH-HCC and ASH-HCC were similar, whereas significantly more patients with ASH-HCC were diagnosed with surveillance compared to those with NASH-HCC. The difference in surveillance possibly resulted from the small sample size, considering the low rate of surveillance and late diagnosis in both etiology groups.²,³ Older age in patients with NASH-HCC may result from the lower surveillance rate, as discussed in Kumar et al.’s study.¹ Moreover, delayed diagnosis of HCC can lead to large tumor and poor outcomes in patients with NAFLD or ALD. Appropriate surveillance strategy for patients with NAFLD or ALD is urgently needed in order to discover more patients at earlier stages, when they are amenable to curative therapies. However, non-cirrhotic patients existed in both groups of Kumar et al.’s study,¹ adding difficulty in selecting the target population for surveillance.

Second, the presence of modifiable lifestyle factors and their impact on the outcome should be studied. In particular, there might have been NASH-HCC patients with nonsignificant (<60 g/day) alcohol consumption in Kumar et al.’s study,¹ which otherwise could be defined as significant, if the amount exceeded >21 (14) standard drinks/week in men (women).⁵ The exact proportion of NASH-HCC patients with alcohol consumption amount between 21 (14) standard drinks/week and <60 g/day is not assessable, due to the lack of information. Likewise, obese ASH-HCC patients (body mass index, ≥25 kg/m²) might also exist in

Abbreviations:
ALD, alcohol-related liver disease; ASH, alcoholic steatohepatitis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis

Editor: Seung Up Kim, Yonsei University College of Medicine, Korea

Received: Mar. 7, 2020 / Accepted: Mar. 9, 2020
the study population. A recent study from a prospective European cohort demonstrated that smoking was an independent predictor of survival in hepatitis B or C-infected HCC patients. In addition, NASH-HCC patients suffer from various comorbidities, such as cardiovascular diseases. Therefore, multiple coexistence of lifestyle risk factors or their comorbid conditions may differ at individual level, and this needs to be verified from the preventive perspective.

Third, the interpretation of etiology-specific outcomes and their comparison requires caution, possibly due to selection bias. Previous studies reported controversial results, especially in terms of survival in NAFLD- or NASH-HCC patients. A prospective multicenter observational study from Italy reported similar survival rates between NAFLD-HCC and hepatitis C virus (HCV)-related HCC groups after patient matching, where the difference in mortality was driven by cardiovascular causes. Another Italian study showed negative effect of alcoholic etiology on survival compared to HCV-HCC, which disappeared after adjustment for confounding factors and stratification by tumor stage. In cohort studies to evaluate and compare etiology-specific outcomes, efforts are needed to adjust the differences in baseline characteristics (e.g., using a propensity score analysis) and to minimize lead-time bias, considering the imbalance in surveillance rates between groups.

In summary, Kumar et al.’s study provided intriguing yet difficult aspects of HCC management, with its epidemiology changing over the last decade. Viral etiologies that underscore the importance of lifestyle-related HCC, such as ALD or NAFLD-HCC, are anticipated to be controlled in the near future. Appropriate surveillance strategy and early diagnosis based on larger-scale, well-designed prospective studies may be the most challenging tasks for improving the outcome.

Authors’ contribution
Conception, manuscript writing and critical revision: HYK and J-FD

Conflicts of Interest
The authors have no conflicts to disclose.

References
Dear Editor,

I read the original article titled "Limited expression of TLR9 on T cells and its functional consequences in patients with nonalcoholic fatty liver disease" by Alegre et al. \(^1\) with great interest. This article studied the role of toll-like receptor (TLR) 9 on T cells in patients with nonalcoholic fatty liver disease (NAFLD) and found positive associations between TLR9 expressions on intrahepatic CD4\(^+\) T cells, necroinflammation, and liver fibrosis. Furthermore, this study revealed associations between TLR9 expression on peripheral CD4\(^+\) and CD8\(^+\) T cells and clinico-pathological alterations of NAFLD, such as body mass index, plasma triglyceride concentration, and aminotransferase activity. In many studies, the pathophysiology of NAFLD has been described as an immune response by either proinflammatory macrophages or T-cell activation via TLRs.\(^2,3\) I also agree that TLRs play a critical role in regulating immune response and are associated with the pathophysiology of various diseases, including NAFLD. Therefore, it is necessary to evaluate TLR-mediated immune response associated with T-cells in NAFLD patients. However, the pathophysiology of NAFLD is not so simple, and one should always consider the fact that various immune responses and signals work in combination.

TLRs are usually expressed on sentinel cells, such as macrophages, and are present in humans in the form of subtypes ranging from TLR1 to TLR10.\(^4\) Although the TLR subtypes play similar roles, each has slightly different functions, and the organs where they are expressed are also slightly different. Therefore, it still remains unclear as to whether TLRs play an important role in NAFLD development. According to previous studies, in addition to TLR9,\(^5\) other TLRs, such as TLR2, TLR4, and TLR5,\(^6,8\) have also been found to play a role in the development of NAFLD. However, this study\(^1\) alone does not reveal which TLRs play a major role in the pathophysiology of NAFLD. In this paper, the question remains as to whether TLR9 is one of the TLRs that play an important role in NAFLD. The authors could have described the role of relative TLR9 in NAFLD more clearly by comparing it with at least one of the other TLRs, such as TLR4.

This article previously stated that TLR9 expression on liver and peripheral T cells is the lowest in patients with simple steatosis, and T cells from patients with simple steatosis induce a limited number of interferon-gamma (IFN\(\gamma\))-producing CD8\(^+\) T cells. However, we should consider whether a decrease in TLR9 expression can explain the reduction in IFN\(\gamma\)-producing cells. IFN\(\gamma\) is known to be an important activator of macrophages and an inducer of class II major histocompatibility complex (MHC) molecule expres-

**Abbreviations:** IFN\(\gamma\), interferon-gamma; MHC, major histocompatibility complex; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; TLR, toll-like receptor

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**Editor:** Seung Up Kim, Yonsei University College of Medicine, Korea

**Received:** Mar. 4, 2020 / **Accepted:** Mar. 4, 2020

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sion. In addition, macrophage activity mediated by IFNγ has been described as an important mechanism for the development of non-alcoholic steatohepatitis (NASH).9,10 In this paper, however, the link between TLR9 and IFNγ is somewhat unclear. Although it is clear that TLR is an important factor regulating IFNγ, IFNγ is itself regulated by several receptors and factors. Without considering various factors that reduce the expression of IFNγ, the reduction in IFNγ levels may be difficult to explain solely as a result of reduced expression of TLR9 in simple steatosis. In fact, studies have shown that various substances secreted from enteric bacteria of NASH patients affect IFNγ production.11 Therefore, the decrease in TLR9 compression and the decrease in IFNγ-producing T cells in this study may have been a phenomenon caused by the complex interactions observed in NAFLD patients. In addition, the possibility of reduced expressions of other TLRs cannot be excluded in patients with simple steatosis.

In conclusion, I also believe that the immune response, including T cells, is important for the pathophysiology of NAFLD, and that the study of TLRs and IFN signaling involved is very important and meaningful. However, this paper did not comment on the effect of other TLRs, and instead focuses on TLR9 only. It also had some limitations in that it did not consider the factors affecting IFNγ besides TLR9. Therefore, the limited TLR9 compression and production of IFNγ, as concluded in this article, could be a logical leap as to whether it actually plays a role in protecting against simple steatosis. As these avenues have not been explored yet, I do not think that the studies mentioned in this article are wrong; however, I believe further research is needed to provide a better reasoning for the efficient role of TLR9 in NAFLD patients.

Conflicts of Interest
The author has no conflicts to disclose.

REFERENCES

9. Rau M, Schilling AK, Meertens J, Hering I, Weiss J, Jurowich C, et al. Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. J Immunol 2016;196:97-105.
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Original article must arranged as follows: (1) title page (2) abstract (250 words or less with a list of 5 or less key words), (3) introduction, (4) materials and methods (or patients and methods), (5) results, (6) discussion, (7) acknowledgements, (8) conflict of interest statement (9) references, (10) tables, and (11) figure legends.

In case of submission of original articles (not applicable for reviews, editorials, and letters), authors should summarize the contents of the article in a concise, pictorial form designed to easily understand main findings of the work described in the article. Graphical abstracts should be submitted as a separate JPG or TIFF files at the online submission step of file upload. The submission of the graphical abstract is mandatory when submitting an original article. Graphical abstracts should be provided as an image with a minimum of 531 × 531 pixels (h × w) using a minimum resolution of 300 dpi. If you are submitting a larger image then please use the same ratio. Please note that your image will be scaled proportionally to fit in the available window, which is a 200 × 500 pixel rectangle.

Review articles

Review articles on selected topics of interest for the readers of the Clinical and Molecular Hepatology and will be solicited by the Editors. Review articles are expected to be clear, concise and updated. The maximum length is 5,000 words. The inclusion of a maximum of 8 high quality tables and/or colored figures to summarize critical points is highly desirable.

Editorials

This section consists of invited brief editorial comments on articles published in the Clinical and Molecular Hepatology. The length of an editorial should not exceed 1,500 words and 1 table or 1 figure is allowed. References should not exceed a maximum of 20.

Case reports

Case reports are not encouraged and will only be accepted if they represent an outstanding contribution to the etiology, pathogenesis or treatment of a specific liver disease. The length of a case report should not exceed 3,000 words. A total number of 2 tables or figures is allowed. References should not exceed a maximum of 10.

Case reports consist of (1) title page, (2) abstract (150 words or less with a list of 5 or less key words) (3) introduction, (4) cases, (5) discussion, (6) references (20 or less), (7) tables and figure legends and (8) figures (5 or less) on separate pages.

Letters to the editor

Letters to the editor should be related to a recent article published in the Clinical and Molecular Hepatology within previous two years, or interesting case report that author wants to report. Letters to the editor must arranged as follows: (1) title page, (2) body (3) references (maximum of 15), and (4) a maximum number of 1 tables or figures is allowed. The length of an letter to the editor should not exceed 800 words. Abstract is not required.

Special topics

Special topics should be no longer than 800 words with 10 or less references.
1. Title page
Provide a concise title. List the full names of all authors and their institutional affiliation. In a multi-authored work involving more than a single institution, indicate individual affiliation by means of superscript Arabic numbers. Indicate a change of address in a similar fashion. List the footnotes to the title page. Provide the contact information for the corresponding author (name, address, telephone number, fax number, e-mail address and Orcid ID), and running title (Less than 50 characters). All abbreviations should be explained in this page (e.g. AFP, alpha fetoprotein; ALT, alanine aminotransferase). Clinical and Molecular Hepatology employs a system to screen plagiarism (CrossRef). When submitting your manuscript to this journal, you accept that your manuscript may be screened for plagiarism against previously published material.

2. Abstract
Abstract of original articles must contain 250 words or less and must be organized as follows: Background/Aims, Methods, Results, and Conclusions. Three to Five keywords should be provided at the end of the abstract. Abstract of case reports must contain 150 words or less in unstructured form.

3. Highlight
Authors of original articles are requested to include “Highlights” which consist of three to four sentences summarizing the originality and main findings of the article. “Highlights” should not exceed 100 words in total. Highlights must be organized in a box and placed after the end of the abstract. The authors are encouraged to include the "Highlights" with initial article submission. When submitting a revised manuscript, the submission of the “Highlights” is mandatory.

4. Introduction
Provide the minimum background information that will orient the general reader. Do not engage in a literature review.

5. Methods
Provide a level of detail such that another investigator could repeat the work. For methods that are used without significant modification, citation of the original work will suffice. Identify and provide references for all the statistical methods used.

6. Results and discussion
Present the major findings of the study in graphical form if practicable. Do not illustrate minor details if their message is adequately conveyed by simple descriptive text. Mention all the tables and figures. In the discussion, concisely present the implications of the new findings for the field as a whole, minimizing any reiteration of the results and avoid repetition of material in the introduction; keeping a close focus on the specific topic of the paper.

7. Acknowledgements
An acknowledgement of persons who made a genuine assistance and provided special reagents may be included. Grant and financial support related with the work should be specifically stated.

8. Authors’ contribution
Based on the ICMJE guidelines for authorship criteria, how each author has contributed to the paper should be clarified (e.g, Conception or design of the work, Data collection, Data analysis and interpretation, Drafting the article, Critical revision of the article, and Final approval of the version to be published).

9. References
References should be numbered in the order they are cited, and the number of reference should be marked in the text by means of a superscript Arabic numerical. Only literature that is published or in press (with the name of the publication) may be numbered and listed; abstracts and letters to the editor may be cited. Cite the names of all authors when there are six or less; when seven or more list the first six followed by et al.
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**11. Abbreviations**

Standard abbreviations not requiring definition are those listed in the Journal of Clinical Investigation. Otherwise, do not abbreviate unless a term is used more than five times in a paper. In this case, the abbreviation should be spelled out, in its first use in the text with the abbreviated form in parentheses, and it should also be listed on the footnote page (see above). Abbreviations used in figures or tables should be defined in the legend. Radiation measurements and laboratory values should be in accordance with the International System of Units (SI) (resources: "SI Units in Radiation Protection and Measurements, NCRP Report no. 82" [August 1985]; "Now Read This: The SI Units Are Here," JAMA 1986;255:2329-2339).

**12. Drug names**

Use generic names. The proprietary name may be mentioned in parenthesis. The names and locations (city and state or country) of manufacturers should be included in parentheses when mentioning proprietary drugs, tools, instruments, software, etc.
13. Tables
Prepare tables on individual sheets of paper, double spaced and numbered consecutively with Arabic numerals in the order of their appearance in the text. The title of tables should be written concisely in clauses and phrases. The first letter of the table title starts with a capital letter. Explain all abbreviations and symbols such as *, †, ‡, §, ‡‡, §§. Do not duplicate the material presented in a figure.

14. Figure legends
Number the figures with Arabic numerals in the order they are mentioned in the text. Provide a title (this should not appear on the figure itself) and sufficient explanation to render the figure intelligible without reference to the text. For any copyrighted material, indicate that permission has been obtained (see Permissions, above). Figure legends should be typed consecutively on a separate sheet of paper.

15. Figures
Illustrations should be sharp and clear. Figure files can be uploaded in the JPG or TIFF formats which authors prefer at a final resolution of not less than 300 dpi. Microscopic pictures should be explained according to the staining method and scaled by the power of magnification. Authors are charged for color figures.

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The journal utilizes blind peer-review in evaluating manuscripts for publication. Submitted papers will be reviewed by at least two referees, and decisions will be available in approximately one months. With respect to the revision and resubmission of manuscripts, it is the journal’s policy to allow a couple of resubmission only, which should be received within 2 months from the time of receipt of the initial review letter. In general, a manuscript requiring more than a couple of revision or returned beyond 2 months will be handled as a new submission. The journal does not have article submission charges. Only when the articles are accepted for publication, domestic corresponding authors are charged for their original articles, brief communications and clinical case reports, which is 6,000 Won (Korean Currency) per page. Page charge is waived for international contributors (corresponding author from outside of Korea), and is reimbursed for domestic contributors.

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Position indicate current status at your affiliation; professor, fellow, resident, student, post doc.
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Please read this checklist carefully to ensure that your manuscript is complete and in compliance with the CMH Guide for Authors.

### 1) General Format

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<td>[2] Is the manuscript double-spaced in an A4-size paper?</td>
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### 2) Abstract

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### 3) Introduction, Methods, Results, Discussion, Acknowledgements, Conflict of Interest Statement, References

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<td>[3] Please state any conflicts of interest.</td>
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<td>[4] All citations in the paper have a complete and accurate reference in the reference list. The number of references in case reports should be 20 or less, and 10 or less in special topics.</td>
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### 4) Tables and Figures

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<td>[4] Figures should be supplied in the JPG or TIFF format at a final resolution of 600 dpi or higher. The number of figures in case reports should be 5 or less.</td>
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