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The Clinical and Molecular Hepatology is an international, peer-reviewed, open-access journal published quarterly in English. The Clinical and Molecular Hepatology 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, the Clinical and Molecular Hepatology 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.

The Clinical and Molecular Hepatology publishes original papers, meta-analysis, letter to editor, case reports, reviews, guidelines, editorials, and liver image and pathology on all aspects of the field of hepatology.

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Reviews

1 Subclinical versus advanced forms of alcohol-related liver disease: Need for early detection
Concepción Gómez-Medina, Luma Melo, David Martí-Aguado, and Ramón Bataller

16 Management of refractory ascites
Florence Wong

33 Wnt signaling in liver regeneration, disease, and cancer
Gengyi Zou and Jae-Il Park

51 Development and prognosis of hepatocellular carcinoma in patients with diabetes
Takuma Nakatsuka and Ryosuke Tateishi

65 Hepatocytes infected with hepatitis C virus change immunological features in the liver microenvironment
Soo-Jeung Park and Young S. Hahn

77 Pathogenesis and treatment of non-alcoholic steatohepatitis and its fibrosis
Kuei-Chuan Lee, Pei-Shan Wu, and Han-Chieh Lin

Editorials

99 The emerging age-pattern changes of patients with hepatocellular carcinoma in Korea
Yuri Cho, Bo Hyun Kim, and Joong-Won Park

102 The rise of non-invasive tools in the diagnosis of portal hypertension: Validation of the Baveno VII consensus
Jeong-Ju Yoo and Sang Gyune Kim

105 Non-invasive tests-based risk stratification: Baveno VII and beyond
Georg Semmler, Mathias Jachs, and Mattias Mandorfer

110 Baveno VII criteria to predict decompensation in compensated advanced chronic liver disease:
Still some shades of grey
Susana G. Rodrigues

113 Moving toward hepatitis B virus functional cure - the impact of on-treatment kinetics of serum viral markers
Lilian Yan Liang, Vincent Wai-Sun Wong, Grace Lai-Hung Wong, and Terry Cheuk-Fung Yip
118 New biomarkers of hepatitis B virus (HBV) infection: HBV RNA and HBV core-related antigen, new kids on the block?  
Young-Suk Lim

Original Articles

120 Hepatocellular carcinoma incidence is decreasing in Korea but increasing in the very elderly  
Young Eun Chon, Seong Yong Park, Han Pyo Hong, Donghee Son, Jonghyun Lee, Eileen Yoon, Soon Sun Kim,  
Sang Bong Ahn, Soung Won Jeong, and Dae Won Jun

135 Baveno-VII criteria to predict decompensation and initiate non-selective beta-blocker in compensated advanced chronic liver disease patients  
Yu Jun Wong, Chen Zhaojin, Giulia Tosetti, Elisabetta Degasperi, Sanchit Sharma, Samagra Agarwal, Liu Chuan,  
Chan Yiong Huak, Li Jia, Qi Xiaolong, Anoop Saraya, and Massimo Primignani

146 Hepatitis B virus pre-genomic RNA and hepatitis B core-related antigen reductions at week 4 predict favourable hepatitis B surface antigen response upon long-term nucleos(t)ide analogue in chronic hepatitis B  
Lung-Yi Mak, Danny Wong, Alison Kuchta, Martina Hilfiker, Aaron Hamilton, Ning Chow, XianHua Mao,  
Wai Kay Seto, and Man-Fung Yuen

Letters to the Editor

163 Letter regarding "Auranofin attenuates hepatic steatosis and fibrosis in nonalcoholic fatty liver disease via NRF2 and NF-κB signaling pathways"  
Yuanbin Liu and Mingkai Chen

165 Letter regarding "Impacts of muscle mass dynamics on prognosis of outpatients with cirrhosis"  
Do Seon Song, U Im Chang, and Jin Mo Yang

168 Letter regarding "COVID-19 vaccine immunogenicity among chronic liver disease patients and liver transplant recipients: A meta-analysis"  
Rujittika Mungmunpuntipantip and Viroj Wiwanitkit

169 Letter regarding "The usefulness of metabolic score for insulin resistance for the prediction of incident non-alcoholic fatty liver disease in Korean adults"  
Hye Won Lee

171 Reply to Letter regarding "Auranofin attenuates hepatic steatosis and fibrosis in nonalcoholic fatty liver disease via NRF2 and NF-κB signaling pathways"  
Seung Min Lee and Dae Won Jun
Reply to Letter regarding “Impacts of muscle mass dynamics on prognosis of outpatients with cirrhosis”
Tae Hyung Kim, Young Kul Jung, and Hyung Joon Yim

Reply to Letter regarding “COVID-19 vaccine immunogenicity among chronic liver disease patients and liver transplant recipients: A meta-analysis”
Ka Shing Cheung, Chiu Hang Mok, Wai Kay Seto, and Man Fung Yuen

Reply to Letter regarding “The usefulness of metabolic score for insulin resistance for the prediction of incident non-alcoholic fatty liver disease in Korean adults”
Jun-Hyuk Lee, Kyongmin Park, Hye Sun Lee, Hoon-Ki Park, Jee Hye Han, and Sang Bong Ahn

Correspondences

Correspondence on Editorial regarding “Impact of nationwide hepatocellular carcinoma surveillance on the prognosis in patients with chronic liver disease”
Won Sohn and Yong-Han Paik

Correspondence on Editorial regarding “Screening and prediction of nonalcoholic fatty liver disease using a peripheral insulin resistance index: Potential benefits and limitations”
Jun-Hyuk Lee, Kyongmin Park, Hye Sun Lee, Hoon-Ki Park, Jee Hye Han, and Sang Bong Ahn

Correspondence on Editorial regarding “Baveno-VII criteria to predict decompensation and initiate non-selective beta-blocker in compensated advanced chronic liver disease patients”
Yu Jun Wong, Sanchit Sharma, Giulia Tosetti, Xiaolong Qi, and Massimo Primignani

Correspondence on Editorial regarding “HBV pgRNA and HBcrAg reductions at week 4 predict favourable HBsAg response upon long-term nucleos(t)ide analogue in CHB”
Lung-Yi Mak, Wai-Kay Seto, and Man-Fung Yuen

Snapshot

Epidemiology and updated management for autoimmune liver disease
Nae-Yun Heo and Haeryoung Kim
Subclinical versus advanced forms of alcohol-related liver disease: Need for early detection

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Alcohol-related liver disease (ALD) consists of a wide spectrum of clinical manifestations and pathological features, ranging from asymptomatic patients to decompensated cirrhosis and hepatocellular carcinoma. Patients with heavy alcohol intake and advanced fibrosis often develop a subacute form of liver failure called alcohol-induced hepatitis (AH). Globally, most patients with ALD are identified at late stages of the disease, limiting therapeutic interventions. Thus, there is a need for early detection of ALD patients, which is lacking in most countries. The identification of alcohol misuse is hampered by the existence of alcohol underreporting by many patients. There are useful biomarkers that can detect recent alcohol use. Moreover, there are several non-invasive techniques to assess the presence of advanced fibrosis among patients with alcohol misuse, which could identify patients at high risk of liver related events or early death. In this review, we discuss differences between early stages of ALD and AH as the cornerstone of advanced forms. A global overview of epidemiological, anthropometric, clinical, analytical, histological, and molecular differences is summarized in this article. We propose that campaigns aimed at identifying patients with subclinical forms can prevent the development of life-threatening forms. (Clin Mol Hepatol 2023;29:1-15)

Keywords: Liver diseases, Alcoholic; Hepatitis, Alcoholic

INTRODUCTION: PREVALENCE OF ALCOHOL-RELATED LIVER DISEASE

Alcohol consumption is one of the most frequent causes of liver disease worldwide. In 2016, according to the World Health Organization, the harmful consumption of alcohol resulted in 3 million deaths (5.3% of all deaths) worldwide and 132.6 million disability-adjusted life years (DALYS) – i.e., 5.1% of all DALYS in that year. Alcohol-related mortality is more prevalent in men and higher than other important diseases such as tuberculosis, acquired immunodeficiency syndrome and diabetes.¹

Diagnosis of alcohol-related liver disease (ALD) requires documentation of an alcoholic use disorder (AUD) and exclusion of other causes of liver disease. ALD consists of a wide spectrum of clinical manifestations and pathological features, from asymptomatic patients to decompensated cirrhosis and hepatocellular carcinoma (Fig. 1).² ³ The early stage of the disease is not well understood, and there is a need to better understand its natural history, risk factors of progres-
sion, and noninvasive diagnosis biomarkers.\textsuperscript{3,5-7} Therefore, underdiagnosis is the rule until severe forms like alcohol-induced hepatitis (AH) develop and mortality is high despite abstinence. In this stage, patients have a terrible prognosis, with short-term mortality rates as high as 50\% at three months due to subsequent organ failure and acute-on-chronic liver failure (ACLF).\textsuperscript{7,8}

The histological and clinical features observed in patients with severe ALD are difficult to replicate in animal models. Additionally, the difficulty in conducting clinical trials in patients with active AUD, the social stigmatization and marginalization of this population, the lack of interest from drug companies and the limitations of current experimental models contribute to a slow progress in ALD management. Thus, the treatment of patients with ALD hasn’t changed much in recent decades, and there aren’t any targeted and personalized medicines available.\textsuperscript{9} To date, the most effective therapy to attenuate the clinical course of ALD and even reverse histological injuries is prolonged alcohol abstinence.\textsuperscript{4}

Identifying risk factors in individuals with AUD that predispose to ALD development is crucial for implementation of public health policies and reduce morbimortality associated to ALD. Several significant biological factors and possible therapeutic targets have been explored in recent translational research. The early recognition of early stages of ALD with subsequent behavioral therapies ought to be encouraged in primary care settings.\textsuperscript{2,4,8-10} For example, alcohol screening questionnaires and basic laboratory test including hepatic profile in high-risk patients.

In this review, we will differentiate between early forms of ALD and AH, the most severe form of ALD, from the epidemiological, anthropometric, clinical, analytical, histological, and molecular stand of points.

**DISEASE STAGES AND NATURAL HISTORY**

ALD includes a wide range spectrum of early and advanced phenotypes. Early phenotypes do not usually have symptoms and can be categorized as subclinical forms. Subclinical stages include fatty liver disease or steatosis, steatohepatitis (ASH) with or without fibrosis and compensated cirrhosis. Although non-invasive biomarkers can have a role in steatosis and fibrosis diagnosis, histological evaluation is needed to define these subclinical stages. Patients with subclinical liver disease and persistent active drinking can end up developing

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**Figure 1.** Spectrum of alcohol-related liver disease. The numbers represent the percentage of patients with progression. From Yamada’s Textbook of Gastroenterology. Permission for their use from the publisher.

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**Abbreviations:**

ACLF, acute-on-chronic liver failure; AH, alcohol-induced hepatitis; AKI, acute kidney injury; ALD, alcohol-related liver disease; ALT, alanine transaminase; ASH, steatohepatitis; AST, aspartate transaminase; AUD, alcoholic use disorder; AUDIT, Alcohol Use Disorders Identification Test; AUROC, area under the receiver operating characteristic; CDT, carbohydrate-deficient transferrin; CIWA, Clinical Institute Withdrawal Assessment for Alcohol; DALYs, disability-adjusted life years; ELF, Enhanced Liver Fibrosis; G-CSF, granulocyte-colony stimulating factor; GGT, gamma-glutamyl transferase; HNF4A, hepatocyte nuclear factor 4 alpha; HSCs, hepatic stellate cells; IL, interleukin; MDB, Mallory-Denk bodies; MDF, Maddrey Discriminant Function; MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; SALVE, Study of Alcohol-related Liver disease in Europe; SWE, shear wave elastography
advanced forms of ALD. Advanced stages are symptomatic and entail a poor prognosis due to liver-related complications. This phenotype includes AH, ACLF and decompensated cirrhosis (Fig. 2).

Steatosis can develop as soon as 3 to 7 days after heavy alcohol consumption. Steatosis is mostly asymptomatic and may be associated with mild elevation of gamma-glutamyltransferase (GGT). It is histologically characterized by macrovesicular fat accumulation, typically located in centrilobular areas. Simple steatosis should not be considered a benign condition since it increases ALD annual mortality up to 6%. In the same line, mortality in biopsy confirmed non-alcoholic fatty liver disease (NAFLD) is also increased among simple steatosis stages.

Although different medical conditions, both ALD and NAFLD show that steatosis carries prognostic implications by itself. Continuous and excessive alcohol drinking may lead, in 10–35%, to the development of ASH, which is characterized by steatosis, hepatocellular damage (i.e., ballooning, Mallory-Denk bodies [MDB]), inflammatory infiltrates—mainly neutrophils—, and different degrees of fibrosis with a pericellular pattern distribution. Approximately 20–40% of patients with ASH will develop progressive fibrosis, of which 8–20% will develop cirrhosis. The risk of cirrhosis is increased in patients with ASH on biopsy as compared with patients with simple steatosis. Once cirrhosis is established, its natural history is characterized by an asymptomatic compensated phase followed by a decompensated phase, marked by the development of overt clinical signs, the most frequent of which are ascites, bleeding, encephalopathy, and jaundice (Fig. 2).

When persistent alcohol intake is maintained, an episode of AH can be developed. Till date, there is no clear explanation why some patients develop this phenotype, nor what are the triggers. It has been speculated that this is due to an increased alcohol consumption, but this has not been firmly demonstrated. AH is associated with high mortality, which can reach 50% in three months, and the median survival time of patients with advanced liver cirrhosis can be as low as 1–2 years.

In a recent study, it was demonstrated that the presence of advanced fibrosis and continued alcohol consumption were the main parameters associated with early mortality in patients with compensated forms of ALD. Moreover, a new parameter has been introduced recently to quantify fibrosis in liver biopsies using digital image analysis, the collagen proportionate area. This parameter predicts liver-related mortality in ALD and hepatic decompensation and/or liver-related death in early/compensated ALD.

Figure 2. Subclinical versus advanced forms of ALD. ALD, alcohol-related liver disease; ASH, steatohepatitis; ACLF, acute-on-chronic liver failure.
SUBCLINICAL ASH VS. AH: CLINICAL, ANALYTICAL AND HISTOLOGICAL FEATURES

Most patients with ALD are identified during late stages of the disease when liver decompensation occurs and when mortality is high despite ethanol cessation. In fact, a global epidemiologic study in 2017 showed that ALD is by far the liver disease etiology that is detected at the latest stages. These results strongly suggest that there is a dire need for the early detection of ALD patients, which currently is almost nonexistent. Recently, a study comparing ASH and AH disease stages has been conducted. This study concluded that patients with AH had higher liver failure and mortality compared to ASH patients (50% vs. 10% 1-year mortality, respectively) with significant different clinical, histological and molecular features.

Although it is assumed that ASH is the harbinger of transition to severe forms of ALD, there are few studies that have assessed clinical and histological features that predict liver disease progression in these patients. Much more research attention has been paid to the severe form of AH. Currently ASH can only be diagnosed with liver biopsy. Similar to NAFLD, there are no signs, symptoms, or biochemical tests that allow the confident diagnosis of ASH. Although asymptomatic, identification of subclinical, molecular and histologic features of ASH would favor its detection worldwide.

The diagnosis of ALD is based on history of heavy alcohol use, typical laboratory markers and clinical features. Compared to similar diseases such as NASH, few patients with ALD undergo a liver biopsy. Most patients with both early and advanced forms are diagnosis without histological assessment. The recent development of a specific fibrosis grading system for patients with ALD could stimulate this field. In patients with AH, a transjugular liver biopsy is justified when there are confounding factors, and the histological changes (i.e., presence advanced fibrosis, polymorphonuclear infiltration, etc.) predict short-term survival.

Some methods to determine alcohol as the major etiology of liver disease include the medical history, surveys, physical examination and laboratory test. It is also important to consider second liver hits that can influence ALD prognosis.

History of alcohol misuse

Criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, are used to define AUD and to determine its severity. Severity is based on the number of criteria a person meets based on their symptoms in the past year. Some surveys can help us to distinguish patients with alcohol abuse. The Alcohol Use Disorders Identification Test (AUDIT) comprises ten questions with a specific scoring system to diagnose alcohol abuse (AUDIT score >8) and alcohol dependence (AUDIT score >20) with 73% sensitivity and 91% specificity vs. 85% sensitivity and 89% specificity respectively. The CAGE questionnaire is also a useful and an easily applied tool. It is more sensitive than the AUDIT to detect alcohol abuse and dependence, but is less effective in recognizing non-severe drinking disorders. However, many patients tend to underreport, particularly in the pre- or post-liver transplant interval, for fear of reprisal by the transplant program. The Clinical Institute Withdrawal Assessment for Alcohol (CIWA) scale assesses the severity of alcohol withdrawal. A randomized, double blind trial published in JAMA in 1994 showed that management for alcohol withdrawal that was guided by the CIWA scale resulted in decreased treatment duration and total use of benzodiazepines.

Physical examination

On physical examination, patients typically have hepatomegaly, which often reflects the combined effects of fatty liver and swelling of hepatocytes due to cell injury-associated protein retention. Signs of chronic alcohol intake (Dupuytren contracture, rhinophyma, etc.) and signs of alcohol withdrawal (tremors, tachycardia, agitation, seizures in severe alcoholic withdrawal syndrome, or delirium tremens) should be screened in primary care for early detection of ALD. Signs of chronic liver disease (spider angioma, palmar erythema and jaundice) and signs of portal hypertension (splenomegaly, ascites, and hepatic encephalopathy) suggest advanced liver disease with underlying cirrhosis. Frequently ALD patients suffer from malnutrition, physical examination may reveal sarcopenia with proximal muscle wasting and decreased grip strength protein.

Laboratory tests

Direct alcohol biomarkers, the most used of which is ethanol detection in urine and/or blood, but they capture only very recent consumption. Alcohol metabolites are clinically...
used as indirect biomarkers of alcohol consumption. Metabolites of alcohol such as urine ethyl glucuronide can reveal alcohol use up to 3–4 days after the last alcohol drink. Sensitivity and specificity of urinary ethyl glucuronide for detection of alcohol use were 89% and 99%, respectively, among patients with ALD before and after liver transplant.\textsuperscript{36,37} Measurement of ethyl glucuronide in hair samples can detect alcohol use for a longer period of up to 1 month.\textsuperscript{38} Other metabolite of alcohol, blood phosphatidylethanol has a half-life of approximately 10–14 days, with sensitivity of 91% and specificity of 77%.\textsuperscript{39} Carbohydrate-deficient transferrin (CDT) has a half-life of 2–3 weeks but its utility is limited by its low sensitivity of 25–50% in several studies and by false-positive results. The levels of CDT may be confounded with increasing disease severity and active smoking. Posttransplant use of %CDT appears to be more accurate, likely due to improved liver function. The CDT combined with GGT has higher sensitivity (75–90%).\textsuperscript{40}

In laboratory test, ALD-induced liver injury indicators are also observed. Increased GGT and aspartate transaminase (AST) levels with typically AST two times greater than alanine transaminase (ALT), macrocytic anemia and thrombocytosis. According to the CLASH study, AH patients presented greater AST/ALT ratio and lower GGT compared to ASH population. In ASH patients, GGT levels were higher, probably reflecting improved preservation of hepatocyte mass (Table 1).

Genetic factors are also involved in the onset, progression, and clinical outcome of ALD. Epidemiological studies conducted among family members and between twins strongly support a genetic component.\textsuperscript{41-43} According to the CLASH study, AH patients showed marked deregulation of genes involved in hepatocyte reprogramming and bile acid metabolism. ASH patients showed a deregulated expression of genes involved in matrisome and immune response.

It is important to consider that in the pathogenesis of liver diseases frequently coexist two different risk factors for liver injury in the same subject, potentially increasing the risk and severity of liver damage. Alcohol is a frequent co-factor in patients with hepatitis C virus infection where it accelerates hepatic fibrosis.\textsuperscript{44,45} Additionally, alcohol has a synergistic hepatotoxic effect with nonalcoholic fatty liver disease, iron overload and other metabolic disorders.\textsuperscript{6,46-49}

<p>| Table 1. Biomarkers in diagnosis and prognosis of patients with of AUD\textsuperscript{14,37} |
|---------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biology</th>
<th>Significance</th>
<th>Cut-off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT</td>
<td>Marker of alcohol liver injury</td>
<td>Unspecific. Liver dysfunction and oxidative stress.</td>
<td></td>
</tr>
<tr>
<td>Transaminase enzymes</td>
<td>Marker of alcohol liver injury</td>
<td>Screening for liver dysfunction in alcohol users.</td>
<td>High specificity if AST/ALT ratio &gt;2</td>
</tr>
<tr>
<td>(ALT, AST)</td>
<td>Marker of alcohol liver injury</td>
<td>Screening for liver dysfunction in alcohol users.</td>
<td></td>
</tr>
<tr>
<td>Blood cell counts</td>
<td>Marker of alcohol liver injury</td>
<td>Unspecific. Normalization in 2–4 months.</td>
<td></td>
</tr>
<tr>
<td>(macrocytic anemia, thrombocytosis)</td>
<td>Marker of alcohol liver injury</td>
<td>Unspecific. Normalization in 2–4 months.</td>
<td></td>
</tr>
<tr>
<td>Urine/blood ethanol</td>
<td>Direct alcohol metabolite</td>
<td>Specific. Recent alcohol intake or alcohol intoxication. Short half-life.</td>
<td>Positive urine EtOH ≥20 mg/dL Positive blood EtOH ≥30 mg/dL</td>
</tr>
<tr>
<td>(EtOH)</td>
<td>Alcohol-derive metabolites</td>
<td>High specificity, low sensitivity for alcohol recent use (2–3 weeks)</td>
<td>CDT &lt;60 mg/L (normal value); 60–100 mg/L (probable alcoholism) and &gt;100 mg/L (very high probability of alcoholism)</td>
</tr>
<tr>
<td>Carbohydrate-deficient transferrin (CDT)</td>
<td>Alcohol-derive metabolites</td>
<td>High specificity, low sensitivity for alcohol recent use (2–3 weeks)</td>
<td>Positive EtG &gt;100 ng/mL Positive EtS &gt;25 ng/mL</td>
</tr>
<tr>
<td>Ethyl-glucuronide (EtG), ethyl sulfate (EtS)</td>
<td>Alcohol-derive metabolites</td>
<td>Recent alcohol intake (3–4 days)High inter-individual variations</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylethanol (PEth)</td>
<td>Alcohol-derive metabolites</td>
<td>Differentiates alcohol- from non-alcohol induced liver disease.</td>
<td>PEth &lt;20 ng/mL (light or no), 20–199 ng/mL (significant) and &gt;200 ng/mL (heavy)</td>
</tr>
<tr>
<td>GGT-CDT combination</td>
<td>Marker of alcohol liver injury + alcohol-derive metabolite</td>
<td>Improves sensitivity and specificity of detecting AUD</td>
<td></td>
</tr>
</tbody>
</table>

AUD, alcoholic use disorder; GGT, gamma-glutamyl transferase; ALT, alanine transaminase; AST, aspartate transaminase.
Non-invasive diagnosis

As mentioned previously, fibrosis is one of the main prognostic factors in ALD. As liver biopsy is not always available, non-invasive methods to assess fibrosis have been developed (Tables 2, 3). Some alternatives are serum biomarkers, such as Fibrotest®, Fibrometer®, Hepascore® and Enhanced Liver Fibrosis (ELF) test. The diagnostic accuracy of these tests is greater than other biomarkers developed for viral hepatitis (i.e., AST to Platelet Ratio Index, Forns index, Fibrosis-4 Index). These simple serum-based parameters obtained from routine liver function tests can distinguish between patients with no fibrosis or advanced fibrosis, but they have limited value to assessed intermediate stages of fibrosis. A combination of any of these tests has not been useful in improving diagnostic performance.

Imaging biomarkers are based on the evaluation of the liver parenchyma stiffness. Elastography measures are based on the speed and elastic wavelength that propagates through the liver tissue. As the stiffness of the liver parenchyma increases related to fibrosis, the elastography value also increase. There are different methods of generating ultra-

Table 2. Biomarkers in diagnosis and prognosis of alcohol-related liver disease

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biology</th>
<th>Significance</th>
<th>Cut-off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfibrillar-associated protein</td>
<td>Marker of fibrogenesis</td>
<td>Early ALD: fibrosis assessment</td>
<td></td>
</tr>
<tr>
<td>Angiopoietin-like</td>
<td>Marker angiogenesis</td>
<td>Early ALD: fibrosis assessment</td>
<td></td>
</tr>
<tr>
<td>Collagen IV, hyaluronic acid</td>
<td>Extracellular matrix turnover</td>
<td>Early-ALD: fibrosis assessment</td>
<td></td>
</tr>
<tr>
<td>PNPLA3</td>
<td>Genetic polymorphism</td>
<td>ALD and AH: disease progression and prognostic Hepatoma development</td>
<td></td>
</tr>
<tr>
<td>HSD17B13, TM6F2</td>
<td>Genetic polymorphisms</td>
<td>ALD: disease progression Prognostic</td>
<td></td>
</tr>
<tr>
<td>Fibrotest</td>
<td>Fibrosis score including alpha-2-macroglobulin, apolipoprotein A1, haptogobulin, Brb, GGT</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 3 risk groups: &lt;0.31, 0.31–0.58, and &gt;0.58</td>
</tr>
<tr>
<td>Fibrometer</td>
<td>Fibrosis score including platelets, prothrombin time, macroglobulin, AST, hyaluronate, age, urea</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 2 risk groups: ≤0.61 (significant fibrosis) and &gt;0.61</td>
</tr>
<tr>
<td>Hepascore</td>
<td>Fibrosis score including age, sex, alpha-2-macroglobulin, hyaluronate, Brb, GGT</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 2 risk groups: ≥0.5 (significant fibrosis) and &lt;0.5 (exclude advanced fibrosis)</td>
</tr>
<tr>
<td>Enhanced liver fibrosis (ELF) test</td>
<td>Fibrosis score including procollagen type III N-terminal peptide, tissue inhibitor of metalloproteinase-1 and hyaluronic acid</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 3 risk groups: &lt;9.8, 9.8–10.5, and &gt;10.5</td>
</tr>
<tr>
<td>FIB4</td>
<td>Fibrosis score including age, ALT, AST, platelets</td>
<td>Fibrosis assessment</td>
<td>Cut-offs between low and intermediate group: 1.3 (for age ≤65 years) and 2.0 (for age &gt;65 years); &gt;2.67 for high-risk group</td>
</tr>
<tr>
<td>APRI</td>
<td>Fibrosis score including ALT, platelets</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 2 risk groups: &lt;0.75 and ≥0.75</td>
</tr>
<tr>
<td>Forn's index</td>
<td>Fibrosis score including age, GGT, cholesterol, platelets</td>
<td>Fibrosis assessment</td>
<td>Cut-offs for 3 risk groups: &lt;4.2, 4.2–6.9, and &gt;6.9</td>
</tr>
</tbody>
</table>

ALD, alcohol-related liver disease; PNPLA3, patatin-like phospholipase domain-containing protein 3; AH, alcohol-induced hepatitis; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; TM6F2, transmembrane 6 superfamily 2; Brb, bilirubin; GGT, gamma-glutamyl transferase; AST, aspartate transaminase; FIB4, fibrosis-4; APRI, AST to Platelet Ratio Index.
sound/elastography waves such as transient elastography and magnetic resonance elastography. Transient elastography could potentially diagnose subclinical liver disease among heavy drinkers, allowing for earlier referral to a specialty liver clinic. Liver stiffness measurement by transient elastography (FibroScan®) closely correlates with the degree of fibrosis, but in studies that did not consider the presence of AH as a possible confounder. Patients with alcohol-related cirrhosis had significantly higher values of liver stiffness than those with viral cirrhosis. Moreover, the cut-off values should be modified in patients with elevated transaminases (AST >200 UI/L). Other confounding factors that can interfere include recent alcohol intake, no compliance with fasting recommendation before the assessment and the presence of cholestasis. A new measurement, the controlled attenuation parameter incorporated into the FibroScan®-device showed a good correlation with steatosis on liver histology. In a prospective, single-etiology cohort study of 462 patients with biopsy-proven ALD and up to 7 years of follow-up, it was found that transient elastography and the ELF test predict liver-related events with excellent prognostic accuracy. They were considered as accurate prognostic markers in patients with early stages of alcohol-related liver fibrosis or compensated cirrhosis. This study also found that cut-offs for transient elastography can be used to separate patients into three groups of distinctly different risks profiles: compared to patients with a liver stiffness below 10 kPa, patients with liver stiffness between 10 and 15 kPa had an 8-fold higher hazard for liver-related events, and those with liver stiffness >15 kPa had a 28-fold higher hazard. In the near future, our group will start an observational study to identify the prevalence of advanced liver fibrosis among patients with excessive alcohol intake using a non-invasive method (transient elastography FibroScan®) and to characterize the main environmental, genetic and epigenetic factors that could influence the development of advanced fibrosis. A new tool has been implemented in conventional ultrasound systems, the shear wave elastography (SWE). It allows to choose the best acoustic window for liver stiffness measurement. In a study carried out in Korea, the area under the receiver operating characteristic (AUROC) of SWE for discriminating ASH from simple steatosis was 0.93 and the AUROC for diagnosing cirrhosis with ASH vs. cirrhosis without ASH was 0.92. Additionally, a cut-off higher than 20 KPa carries prognostic information. Although magnetic resonance elastography has the best diagnostic accuracy for liver fibrosis detection, its’ use is not widely available in clinical settings because it requires specific software and an external device. A recent study carried out by Chen et al. has also identified some prognostic factors based on computed tomography radiomics (texture, liver surface nodularity and steatosis measurements) which were associated with reduced 90-day

Table 3. Biomarkers in diagnosis and prognosis of patients with AH

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Biology</th>
<th>Significance</th>
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<tr>
<td>Keratin-18, 30/M65</td>
<td>Marker of necro-apoptosis hepatocytes</td>
<td>Diagnostic and prognostic</td>
</tr>
<tr>
<td>Lipoprotein Z</td>
<td>Abnormal free cholesterol-enriched LDL-like particle. Hepatotoxic.</td>
<td>Prognostic</td>
</tr>
<tr>
<td>Cytolysin</td>
<td>Product Enterococcus faecalis</td>
<td>Prognostic</td>
</tr>
<tr>
<td>Bacterial DNA</td>
<td>Product gut bacteria</td>
<td>Prognostic and infection development</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Marker of HNF4A function</td>
<td>Prognostic</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Product gram-negative bacteria</td>
<td>Prognostic and therapeutic response</td>
</tr>
<tr>
<td>IL-6, IL-8, IL-20</td>
<td>Inflammatory cytokines</td>
<td>Prognostic</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Extracellular protein marker</td>
<td>Prognostic</td>
</tr>
<tr>
<td>miR122, miRNA155, miRNA192</td>
<td>Epigenetic regulators</td>
<td>Prognostic</td>
</tr>
<tr>
<td>PNPLA3</td>
<td>Genetic polymorphism</td>
<td>ALD and AH: disease progression and prognostic Hepatoma development</td>
</tr>
</tbody>
</table>

AH, alcohol-induced hepatitis; LDL, low-density lipoprotein; HNF4A, hepatocyte nuclear factor 4 alpha; IL, interleukin; ALD, alcohol-related liver disease; PNPLA3, patatin-like phospholipase domain-containing protein 3.
and overall transplant-free survival in AH.

**Histology assessment**

Histologically, AH is associated with ballooned hepatocytes, MDB, lobular polymorphonuclear neutrophils and pericellular and sinusoidal fibrosis (in “chicken wire” appearance). The histological features of ASH are similar from those described in NASH. 62

According to the CLASH study, AH presented histologically more advanced fibrosis, MDB, bilirubinostasis, severe neutrophil infiltration and progenitor cell expansion than ASH. AH was characterized by profound ductular reaction, which is not seen in early asymptomatic phases and is associated with poor prognosis. Another study carried out by Ventura-Cots et al. 63 revealed through histological and molecular profiling that ductular reaction is a driver of portal hypertension in patients with AH.

The SALVE (Study of Alcohol-related Liver disease in Europe) Histopathology Group developed and validated a grading and staging system for the clinical and full histological spectrum of ALD and evaluated its prognostic utility in a multinational cohort of 445 patients. 27 SALVE grade was described by semiquantitative scores for steatosis, activity (hepatocellular injury and lobular neutrophils) and cholestasis. The histological diagnosis of ASH due to ALD (histological ASH) was based on the presence of hepatocellular ballooning and lobular neutrophils. Severe cirrhosis and histological ASH were identified as independent predictors of short-term survival in decompensated ALD, and decomposition-free survival in compensated ALD.

Altamirano et al also presented a histologic scoring system that relates to the prognosis in patients with AH. 23 The authors identified histologic features associated with AH disease severity and proposed a semiquantitative scoring system, the “Alcoholic Hepatitis Histologic score”. Four histologic features were combined to create the final score: fibrosis stage, bilirubinostasis, polymorphonuclear infiltration, and megamitochondria. The degree of fibrosis and the presence of bilirubinostasis were positively associated with higher short-term mortality. On the other hand, mild polymorphonuclear infiltration and absence of megamitochondria were associated with poorer outcome in these patients. Low, moderate, and high score was associated with a short-term mortality of 3%, 19%, and 51%, respectively. The prognostic assessment of this score to predict short-term mortality compared favorably with well-validated, non-invasive scoring systems for AH such as the Model for End-Stage Liver Disease (MELD). Further, the histologic score was able to provide additional prognostic information in AH patients with low MELD scores. In patients with a MELD score of <21, a cutoff value of 5 points in the histologic score differentiated two subgroups with different 90-day survival (94% vs. 72%). However, a recent study showed that this score is not predictive of short-term survival in patients with severe AH. Further studies should evaluate whether the prognostic value of histologic parameters differs according to the severity of liver disease. 64

**AH**

AH is clinically defined as abrupt onset of progressive jaundice and liver-related complications with hyperbilirubinemia (>3 mg/dL), AST/ALT ratio >1.5 with levels of AST >1.5 times the upper limit of normal but <400 IU/L; and heavy alcohol drinking until 4 weeks before onset of symptoms and absence of other causes of liver disease (10–20%). 65

To date, multiple scoring systems have been developed to predict short-term prognosis in patients with AH. One of the most validated is the Maddrey Discriminant Function (MDF), which includes prothrombin time and total bilirubin, and where severe AH is defined by a score ≥32. 66 Other scoring systems include the MELD score, 67 the Glasgow Alcoholic Hepatitis score 68 and the age, serum bilirubin, INR and serum creatinine. 69,70 MELD score incorporates renal function, as a major determinant of outcomes in AH patients. A MELD score >20 has been proposed as definition of severe AH. 62 A recent large worldwide study showed that MELD is the best scoring system to predict mortality in alcohol-associated hepatitis. 72 Another relevant prognostic tool is the Lille score, assessed after 4–7 days of corticosteroid therapy. A Lille score <0.45 predicts a good response to corticosteroids and continuing prednisolone for 4 weeks is recommended. 73

Acute kidney injury (AKI) is a common complication in patients with AH and negatively impacts short-term survival. 74,75 Therefore, serum creatinine should be screened in all patients with AH. A recent study carried out by Fernandez-Carrillo et al. 76 found out that AKI in the setting of AH is characterized by a higher urine potassium concentration. Among the biomarkers considered in the study, urine NGAL, IGFBP-7, KIM-1, LFABP, as well as TIMP-1 and TIMP-2 discriminated AKI.
NGAL predicted 3-months survival (AUROC 0.72). Among patients with AH. Among all studied biomarkers, was no correlation between serum and urine IL-18 levels not sustained at 90 or 180 days.

Patients with non-severe AH, defined as a MELD score ≤20 or MDF <32, have a low risk of short-term mortality. The 5-year mortality of decompensated patients with ASH and an MDF <32 is about 50%.

Regarding the AH treatment, the only therapy with proven benefit is alcohol abstinence. There are multiple scores to predict alcohol relapse after an AH episode. A recent study carried out by Clemente et al., discriminate different risk groups for early alcohol relapse after an episode of AH. The higher group risk were patients under 44 years of age, MELD >21, no cirrhosis or psychiatric disease diagnosis, no relationships and unemployed. A therapy that has shown likely benefit is corticosteroids. Prednisolone (40 mg/day) given orally should be considered to improve 28-day mortality in patients with severe AH (MDF ≥32) without contraindications to the use of corticosteroids. However, survival benefit was not sustained at 90 or 180 days. The Lille score should be used to reassess prognosis, identify non-responders, and guide treatment course after 7 days of corticosteroids. Other therapies with potential benefit are N-acetylcysteine and Granulocyte-colony stimulating factor (G-CSF). In patients with steroid non-responsive severe AH (day 7 Lille score >0.45), the administration of G-CSF reduces the disease severity and 90-day mortality. According to a recent presentation at the International Liver Congress 2021 carried out by Louvet et al., amoxicillin/clavulanate plus prednisolone in severe AH do not improve survival at 2 months. Future treatment to consider are IL-1R antagonist anakinra, fecal transplantation, DUR-928, and IL-22 agonist F-652. It should also be considered the early liver transplant for severe AH not responding to medical therapy, has been demonstrated that improves survival compared to patients without transplant.

SUBCLINICAL ASH VS. AH: MOLECULAR PROFILING

The cellular and molecular mechanisms of ALD are multifaceted, complex, and poorly understood in part due to the lack of clinical and histological replication in animal models. The majority of mechanistic investigations uses animal models to identify several molecular drivers of intermediate ALD (steatosis and mild inflammation), but not the severe fibrosis and cholestasis that are seen in advanced ALD.

Alcohol is processed into acetaldehyde in the liver forming proteins and DNA adducts, promoting lipid peroxidation, glutathione depletion, and mitochondrial damage. These adducts also act as antigens, causing lymphocyte migration to the liver and activating the adaptive immune response. Kupffer cells release anti-inflammatory cytokines (IL-10) and hepato-protective factors (IL-6) that help to prevent hepatocellular damage caused by alcohol.

On top of that, acute alcohol intake or binge drinking also increase serum levels of bacterial products. Alcohol increases gut permeability and bacterial product translocation into the portal circulation, leading to the generation of proinflammatory cytokines such tumor necrosis factor, which contribute to hepatocellular damage. A variety of proinflammatory cytokines, such as IL-1, IL-8, osteopontin, CXCL1, CXCL4, CXCL5, and CXCL6, are up-regulated during alcoholic liver injury and also contribute to neutrophil recruitment. Recently, there is an increasing amount of evidence suggesting the gut-liver axis and bacterial dysbiosis as a major factor in ALD, potentially becoming a target for therapy. There are major imbalances in the gut barrier that facilitates pathogen-associated molecular patterns, gut-derived bacterial products that trigger mostly hepatic stellate cells (HSCs) and Kupffer cells, to translocate into the portal circulation. The more severe the AH onset gets, the more gut dysbiosis and bile-acid disbalance is seen.

The major pathogenic and prognostic event in the development of ALD is the progression of fibrosis. Alcohol abuse can cause liver fibrosis by extracellular buildup of collagen and other matrix proteins. It promotes collagen expression in HSCs and, when coupled with other biological components, forms a variety of adducts that keep HSCs active. Neutrophils, injured hepatocytes, and activated Kupffer cells can also stimulate HSCs by releasing profibrogenic mediators such as transforming growth factor, platelet-derived growth...
factor, IL-8, angiotensin II, and leptin. HSC profibrogenic signaling pathways are also stimulated by reactive oxygen species, resulting in fibrogenesis enhancement through modulation of angiogenesis. The endoplasmic reticulum and mitochondrial-derived glutathione continuously neutralize the reactive oxygen species produced by the metabolism of alcohol. However, whenever the body is exposed chronically to alcohol, mitochondrial-derived glutathione becomes depleted, and the reactive oxygen species interact with iron and ethanol forming reactive metabolites responsible for lipid peroxidation of cell membranes.

Activated HSCs are destroyed by natural killer cells, which release interferon, cause HSC cell cycle arrest and apoptosis. Thus, alcohol reduces the ability of natural killer cells to fight fibrosis and liver regeneration becomes inefficient. In advanced cirrhotic ALD, chronic alcohol exposure impairs liver regeneration by inhibiting DNA synthesis in mature hepatocytes. Thus, along with hepatocyte dedifferentiation, the liver regeneration impairment is a key event leading to liver failure in ALD.

The proliferation mechanisms of adult hepatocyte are entirely substituted by massive ductular cell proliferation and an intensification in the number of hepatocytes expressing markers from progenitor cell. Our group has shown recently that patients with AH have a hepatocyte nuclear factor 4 alpha (HNF4A) transcriptomic footprint. Liver-enriched transcription factors, such as HNF1A, RXRA, and FOXA1, were shown to be downregulated, while HNF4A P2 variants, characteristically expressed throughout fetal liver development, are expressed in AH patients. Thus, the restoration of HNF4A function can potentially be a therapeutic target for severe ALD (Fig. 3).

CONCLUSIONS

The prevalence of AUD and ALD is increasing in a global manner. ALD includes a wide range spectrum of early and advanced phenotypes. Most patients are seen at advanced stages of the disease when an episode of AH and/or clinical

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**Figure 3.** Pathogenesis of alcohol-related liver disease and alcohol-induced hepatitis. Excessive alcohol intake cause Kupffer cells and Stellate cells activation along with changes in the microbiome and increased gut permeability. The resulting liver damage, hepatic and systemic inflammation and liver fibrosis are responsible for the dominant clinical consequences seen in AH patients. In addition, expansion of immature progenitor cells leads to impaired regeneration and HNF4a P2 variants are seen expressed in AH. LPS, lipopolysaccharide; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor kappa B; TNF, tumoral necrosis factor; ROS, reactive oxygen species; HSC, hepatic stellate cell; TGF-β, transforming growth factor-β; STAT, signal transducers and activators of transcription; SIRS, systemic inflammatory response syndrome; HNF4A, hepatocyte nuclear factor 4 alpha; AH, alcohol-induced hepatitis.

decompensations are developed. Campaigns for early detection of asymptomatic or subclinical forms are urgently needed. ALD is characterized by profound fibrogenesis even in subclinical forms. Compared to compensated ALD, AH is characterized by profound reprogramming of hepatocytes with features of de-differentiated and cholangiocytes. Translational studies have identified novel targets for the design of therapeutic clinical trials.

**Unmet needs for future research**

Scientific consensus conference to better define subclinical stages of ALD. Cost-effective measures and public health policies to reduce alcohol consumption. Early detection of AUD and concomitant ALD is urgently needed. As the underlying mechanisms of subclinical ASH remain elusive, further research is needed to clarify druggable molecular drivers as well as prognostic biomarkers. Effective and safer therapies for patients with ALD are still needed.

**Authors’ contribution**

CGM and LM wrote the manuscript and prepared the figures and tables. DMA and RB revised the manuscript. All the authors read and approved the final version.

**Conflicts of Interest**

The authors have no conflicts to disclose.

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Management of refractory ascites

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The development of refractory ascites in approximately 10% of patients with decompensated cirrhosis heralds the progression to a more advanced stage of cirrhosis. Its pathogenesis is related to significant hemodynamic changes, initiated by portal hypertension, but ultimately leading to renal hypoperfusion and avid sodium retention. Inflammation can also contribute to the pathogenesis of refractory ascites by causing portal microthrombi, perpetuating the portal hypertension. Many complications accompany the development of refractory ascites, but renal dysfunction is most common. Management starts with continuation of sodium restriction, which needs frequent reviews for adherence; and regular large volume paracentesis of 5 L or more with albumin infusions to prevent the development of paracentesis-induced circulatory dysfunction. Albumin infusions independent of paracentesis may have a role in the management of these patients. The insertion of a covered, smaller diameter, transjugular intrahepatic porto-systemic stent shunt (TIPS) in the appropriate patients with reasonable liver reserve can bring about improvement in quality of life and improved survival after ascites clearance. Devices such as an automated low-flow ascites pump may be available in the future for ascites treatment. Patients with refractory ascites should be referred for liver transplant, as their prognosis is poor. In patients with refractory ascites and concomitant chronic kidney disease of more than stage 3b, assessment should be referred for dual liver-kidney transplants. In patients with very advanced cirrhosis not suitable for any definitive treatment for ascites control, palliative care should be involved to improve the quality of life of these patients. (Clin Mol Hepatol 2023;29:16-32)

Keywords: Ascites; Transjugular intrahepatic portosystemic stent shunt; Liver transplantation; Albumin; Paracentesis

INTRODUCTION

The development of ascites in the natural history of cirrhosis heralds the onset of decompensation. More contemporary data from the United Kingdom suggest that decompensation occurs at the rate of 31% in the first year after the diagnosis of cirrhosis, and thereafter at the rate of 5–7% per annum, with ascites being the most common mode of decompensation.1 Ascites is usually responsive to diuretic therapy at the initial stage. However, with the progression of the cirrhotic process, renal sodium retention becomes more avid and increasing diuretic doses are required to control the ascites. Ultimately, the patient either develops complications to the diuretics or the ascites is no longer responsive to the diuretics. The patient is said to have refractory ascites (RA) and some form of second-line therapy will need to be instituted. Approximately 10% of patients with cirrhosis and ascites have RA at any given time. In addition to the usual complica-
tions associated with the presence of ascites such as the risk for the development of spontaneous bacterial peritonitis, electrolyte abnormalities, or renal dysfunction, the presence of RA is associated with its own unique problems such as a constant sense of fullness, decreased appetite, the development of various hernias, nutritional deficiencies, and sarcopenia. Therefore, patients with RA have a very poor quality of life. Older literature has indicated a 1-year mortality for patients with RA to be at 50%, although more recent reports have indicated a slightly improved prognosis, but the mortality is still in excess of 20% at 1 year.

DEFINITION OF RA

Tense ascites can be recurrent or refractory. Recurrent ascites is ascites that recurs at least three times a year despite dietary sodium restriction and diuretic therapy. It may be a forerunner of RA. RA is defined as ascites that cannot be mobilized or the early recurrence of which (after a large volume paracentesis [LVP]) cannot be prevented by medical therapy. RA can be divided into two subtypes: diuretic resistant or diuretic intolerant. Table 1 details the diagnostic criteria for both subtypes.

PATHOPHYSIOLOGY OF RA

Patients with decompensated cirrhosis have significant hemodynamic changes, initiated by architectural distortion of the liver related to cirrhosis. The laying down of fibrous scar tissues and nodular formation within the liver provides the fixed component of obstruction to portal flow, while stellate cell activation furnishes the dynamic component of increased resistance to portal flow. Stellate cells themselves also produce extracellular matrix and collagen, adding to the fixed component of the increase in intrahepatic resistance as the liver cirrhosis progresses. Microthrombi formation within the

Table 1. Diagnostic criteria for refractory ascites

<table>
<thead>
<tr>
<th>Criteria</th>
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<tr>
<td>Refractory ascites</td>
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<tr>
<td>Ascites that cannot be mobilized or the early recurrence of which (i.e., after therapeutic paracentesis) cannot be satisfactorily prevented by medical therapy</td>
</tr>
<tr>
<td>A) diuretic resistant</td>
</tr>
<tr>
<td>The development of refractory ascites is due to lack of response to dietary sodium restriction and maximal doses of diuretics</td>
</tr>
<tr>
<td>B) diuretic intractable</td>
</tr>
<tr>
<td>The development of refractory ascites is due to the development of diuretic-induced complications* that precludes the use of effective doses of diuretics</td>
</tr>
<tr>
<td>Duration of treatment</td>
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<tr>
<td>Maximum doses of diuretic + adherence to a low sodium diet of ≤88 mmol/day for ≥1 week</td>
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<tr>
<td>Maximum diuretic doses</td>
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<tr>
<td>Either spironolactone 400 mg/day or amiloride 30 mg/day plus furosemide 160 mg/day</td>
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<tr>
<td>Lack of response</td>
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<td>Mean weight loss of &lt;0.8 kg over 4 days and daily urinary sodium excretion less than the daily sodium intake</td>
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<tr>
<td>Early ascites recurrence</td>
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<tr>
<td>Re-appearance of grade 2 or moderate ascites with moderate symmetrical abdominal distention, or grade 3 with massive ascites with marked abdominal distention within 4 weeks of initial mobilization</td>
</tr>
<tr>
<td>Diuretic induced complications*</td>
</tr>
<tr>
<td>Renal impairment, hyponatremia, hypo- or hyperkalemia, hepatic encephalopathy</td>
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</table>

Adapted from Salerno et al.5

*Renal impairment: increase of serum creatinine by >100% to a value >133 μmol/L (2 mg/dL) in patients with ascites responding to treatment. Hyponatremia: decrease of serum sodium by >10 mmol/L to a serum sodium of <125 mmol/L. Hypo-or hyperkalemia: change in serum potassium to <3 mmol/L or >6 mmol/L despite appropriate measures. Hepatic encephalopathy: development of encephalopathy in the absence of any other precipitating factor.

Abbreviations:

ACLF, acute-on-chronic liver failure; alfapump, automated low flow ascites pump; BNP, brain natriuretic peptide; CKD, chronic kidney disease; EABV, effective arterial blood volume; FIPS, Freiburg Index of Post-TIPS survival; HE, hepatic encephalopathy; LVP, large volume paracentesis; MELD, Model of End-stage Liver Disease; NSBB, non-selective beta-blocker; PPCD, post-paracentesis circulatory dysfunction; PTFE, polytetrafluoroethylene; RA, refractory ascites; TIPS, transjugular intrahepatic porto-systemic stent shunt
intrahepatic vasculature can add to the distortion of the liver architecture by causing areas of parenchymal extinction.\textsuperscript{5} Another process that contributes to the progressive increase in portal hypertension in cirrhosis is the development of collateral vessels. There is angiogenesis driven by vascular endothelial growth factor, augmenting the splanchnic capacitance, leading to increased portal flow,\textsuperscript{6} perpetuating the portal hypertension.

The development of portal hypertension has many downstream effects. Firstly, the distension of the splanchnic vessels increases the shear stress on the vessels, and this leads to the production of various vasodilators including nitric oxide. As a result, splanchnic vasodilatation occurs. Some of these excess splanchnic vasodilators can be transferred to the systemic circulation via portosystemic shunts, causing systemic vasodilation. The resultant relative insufficient effective arterial blood volume (EABV) leads to the activation of various vasoconstrictor systems in an attempt to reduce the extent of the splanchnic and systemic vasodilation and to stimulate renal sodium and water retention to increase the intravascular volume. However, the presence of portal hypertension will preferentially localize the excess fluid into the peritoneal cavity as ascites, leaving the central circulation relatively deficient in EABV.

Another downstream effect of portal hypertension is the disruption of the gut vascular barrier related to venous congestion from splanchnic vasodilatation and splanchnic neoangiogenesis. The increased permeability of the gut results in a rise in the translocation of gut bacteria. Many of these bacterial products have vasodilatory properties themselves; contributing to the splanchnic vasodilatation. Other components of bacterial products can stimulate the innate immune system, leading to systemic inflammation. Within the liver, the pro-inflammatory milieu promotes further fibrosis; within the splanchnic circulation, inflammation promotes splanchnic thrombosis,\textsuperscript{7,8} further aggravating the portal hypertension, thereby perpetuating the above-mentioned portal hypertension-related hemodynamic changes (Fig. 1).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{ascites_pathophysiology.png}
\caption{Pathophysiology of ascites formation. DILI, drug induced liver injury; DAMP, damage associated molecular pattern; PAMP, pathogen associated molecular pattern; EABV, effective arterial blood volume.}
\end{figure}
As the cirrhotic process progresses, and the portal hypertension increases, the above changes become more severe, and the sodium retention becomes more avid, while the renal circulation becomes more vasoconstricted. Ultimately, altered renal blood flow sets in, renal hypoperfusion ensues, leading to the development of chronic renal insufficiency, or what was previously known as type 2 hepatorenal syndrome, and the ascites becomes refractory to diuretic therapy.

**MANAGEMENT OF RA**

The management of patients with RA should follow a stepwise approach, starting with sodium restriction, and LVP. Judicious use of medications could avoid further complications.

In the appropriate patients, the insertion of a transjugular intrahepatic portosystemic stent shunt (TIPS) should be considered. All patients with RA should be assessed for liver transplant (Fig. 2).

**Dietary sodium and fluid restriction**

Dietary sodium restriction is required at all stages of ascites including those with RA, as it reduces the rate of ascites accumulation. It is recommended that daily sodium intake should be limited to 88 mmol or 2 g per day. Counselling with a dietitian is helpful, as is frequent reviews of food diary, especially in patients who are accumulating ascites at a rapid rate. Information on where to purchase low sodium food items and advice on low sodium recipes are other measures that

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**Figure 2.** Suggested treatment algorithm of refractory ascites. ICA, International Ascites Club; LVP, large volume paracentesis; PPCD, postparacentesis circulatory dysfunction; TIPS, transjugular intrahepatic portosystemic stent shunt; alfapump, automated low flow ascites pump; CKD, chronic kidney disease. *Second line therapies are: LVP, TIPS or alfapump.
can improve compliance with sodium restriction. Some patients who have been labeled as having RA can lose their ascites and start responding to diuretics again once they adhere to their sodium restriction, especially in patients whose daily renal sodium excretion is more than 88 mmol/day.

Fluid restriction is not required in patients with RA. It is difficult to enforce and is not practical. Fluid restriction is only useful when the fluid intake is less than the urine output, which in patients with RA is often around 500 mL/day. In patients who have hyponatremia with serum sodium of ≤125 mmol/L, it is recommended that some fluid restriction be instituted. However, the level of serum sodium that should initiate fluid restriction has not been well defined.

**Calculating the sodium balance**

This is important in determining compliance with dietary sodium restriction, especially in patients who are rapidly gaining weight after a LVP. A 24-hour urine collection to measure the renal sodium output and a weight chart are required. A 24-hour urine collection is preferred to a spot urine sample as it is more accurate. In patients who are prescribed an 88 mmol daily sodium restriction diet, and who are excreting no urinary sodium at all, the daily sodium accumulation is 88 mmol/day or 616 mmol/week. Since the ascitic sodium concentration is the same as serum sodium concentration, the weekly ascites accumulation is 616 mmol/week ÷ 140 mmol/L or 4.4 L/week. Any patient who is requesting a weekly LVP of more than 4.4 L is clearly non-compliant with dietary sodium restriction, and repeat dietary counselling is needed (Fig. 3). The accumulation of ascites is usually a little less, as there is insensible loss of sodium through the respiratory tract. Frequently, a food record is very revealing, as many patients regard sodium restriction as “just not adding salt at the table” without realizing that many prepared food items are high in sodium. Patients who are excreting more than 88

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**Figure 3.** Calculating the sodium balance. LVP, large volume paracentesis; Na, sodium; TIPS, transjugular intrahepatic portosystemic stent shunt. *Renal impairment as indicated by increase of serum creatinine by >100% to a value of >133 µmol/L or 2 mg/dL; or hyponatremia with a decrease in serum sodium of >10 mmol/L to a value of <125 mmol/L; or hypokalemia to a value of <3.0 mmol/L; or hyperkalemia to a value of >6.0 mmol/L; or the development of hepatic encephalopathy in the absence of any other precipitating factors.
mmol of sodium per day should be losing weight while on an 88 mmol sodium intake per day, as they should be in a negative sodium balance. If this is not happening, the dietary re-education is needed.  

**Albumin infusions**

Regular albumin infusions have been advocated for the management of patients with decompensated cirrhosis. Indeed, in patients with uncomplicated ascites who were still responding to diuretic therapy, the use of regular albumin infusions, initially 40 g twice weekly for 2 weeks, then 40 g weekly for a total of 18 months, has been shown to improve their overall survival, especially in patients whose serum albumin was maintained at a minimum of 40 g/L. However, for patients with more advanced cirrhosis who were on the liver transplant waiting list, the use of regular albumin infusions at a dose of 40 g every 2 weeks plus midodrine did not impact their probability of developing complications nor their survival. In the only randomized controlled trial including 70 patients with cirrhosis and RA, 45 patients were randomized to receive 40 g of albumin twice weekly. There was a significant reduction in the 24-month hospital admissions for complications of cirrhosis and mortality. This suggests that regular albumin infusions may be beneficial for these patients. However, further supportive randomized controlled trials are needed before regular albumin infusions can be recommended as the standard of care for patients with cirrhosis and RA. It also appears that the dosing and frequency of infusions may be important to achieve positive results.

**The use of non-selective beta-blockers (NSBBs)**

NSBBs are the cornerstone in the management of portal hypertension in cirrhosis. The blocking of β1 adrenergic action reduces heart rate and hence cardiac output by 20%; the blocking of β2 adrenergic action in the splanchnic vasculature allows unopposed adrenergic action, causing splanchnic vasoconstriction, and hence reduced portal inflow including that from collateral vessels by about 15%. Therefore, the total reduction in portal venous flow with NSBB use is approximately 35%. Labetalol and carvedilol are 2 NSBBs that also have α1 adrenergic blocking effects, and therefore can cause intra-hepatic vasodilatation, with further reduction in portal pressure. The use of NSBB in patients with compensated cirrhosis and clinically significant portal hypertension (hepatic venous pressure gradient ≥10 mmHg) has been shown to significantly reduce the likelihood of decompensation or death. However, the use of NABBs in patients with ascites is more controversial. The initial studies certainly indicated that the use of NSBBs in patients with ascites, especially those with RA, was associated with increased complications and mortality. Subsequent studies showed that NSBB use in patients with ascites, including those with RA had no impact on the development of renal dysfunction or mortality. There were also less bacterial infections with NSBB use. In fact, the withdrawal of NSBB was associated with an increase in the likelihood of variceal bleeding, bacterial infections, and the development of renal dysfunction, as well as an increase in hospitalization rate and mortality. In patients with acute-on-chronic liver failure (ACLF), many of whom had RA, the use of NSBB was thought to be associated with a reduction in ACLF grade. These seemingly contradictory findings may be related to significant heterogeneity among the various studies.

The recent detailed evaluation of the cardiovascular effects of NSBB use in advanced cirrhosis has shed some light to guide the use of NSBB in patients with RA. These patients with their significant arterial vasodilatation are critically dependent on adequate cardiac systolic function and sympathetic hyper-activity to maintain renal perfusion. Therefore, the use of NSBB may impair cardiac systolic function and reduce the renal perfusion pressure to the point that is below the threshold of renal blood flow autoregulation. That is, the kidneys are no longer able to adjust the renal perfusion in response to a fall in the perfusion pressure. Therefore, patients with RA who are taking NSBB are at risk for the development of renal dysfunction including hepatorenal syndrome. The guidance from the 2021 American Association for the Study of the Liver on the management of ascites suggests that NSBBs may be withheld in patients with hemodynamic abnormalities as indicated by low systolic blood pressure <90 mmHg, hyponatremia with serum sodium <130 mmol/L, or the presence of acute kidney injury. NSBBs might be re-introduced if circulatory dysfunction improves with improvement of these parameters. Carvedilol is not recommended for patients with RA as it causes more systemic hypotension due to its additional adrenergic blocking effects.
Large volume paracentesis (LVP)

LVP arbitrarily has been defined as removal of ascites of >5 L. Because LVP does not correct the underlying pathophysiology of ascites formation, ascites recurs soon after a session of LVP. This is because the loss of ascites through LVP is associated with a reduction in the intra-abdominal pressure, and this tends to exaggerate the pressure difference between the cirrhotic liver and the abdominal cavity, which encourages the rapid refilling of the abdominal cavity. Therefore, repeat LVPs are usually required in the management of these patients. Repeat LVPs have been shown to be safe and effective in the management of RA in cirrhosis, and is associated with lower incidence of electrolyte abnormalities, renal dysfunction, and hemodynamic instability when compared to continued diuretic use. However, the redistribution of the circulatory volume to refill the abdominal cavity can lead to a further reduction in the EABV, increasing the likelihood of developing further renal dysfunction, dilutional hyponatremia, and risk for mortality, a condition known as post-paracentesis circulatory dysfunction (PPCD). Therefore, volume replacement with colloid solutions such as albumin has been recommended following LVP to prevent PPCD. In general, the higher the volume of LVP, the more likely the patient is to develop PPCD. There has never been a dose response study for albumin use for LVP and the literature has reported various doses of albumin being used with LVP. Expert opinion suggests an albumin dose of 6–8 g/L of ascites removed, although reduced dose of 4 g of albumin/L of ascites removed was equally effective in the prevention of PPCD. A further study showed that by providing a higher amount of albumin of 9.0±2.5 g/L of ascites removed and limiting LVP to 8 L can prevent the development of renal dysfunction despite the presence of PPCD. Survival was also not affected over a mean follow-up of 2 years in those who developed PPCD.

It has been suggested that paracenteses of <5 L do not require any intravascular volume replacement, as these small paracenteses are not associated with significant disturbance of systemic and renal hemodynamics. However, in patients with ACLF, albumin use with small volume paracentesis has been shown to reduce the incidence of PPCD together with its attendant complications such as acute kidney injury, hyponatremia, and high mortality. This is because albumin, with its volume expanding, anti-inflammatory and immune modulatory properties, can significantly reduce the heightened inflammation and severely deranged hemodynamics that are commonly observed in patients with ACLF.

Finally, the presence of coagulopathy should not be a contra-indication to LVP, as minimal bleeding was reported with LVP even in patients who had an PT-INR of >1.5 and a platelet count of <50×10⁹/L. Therefore, the infusion of platelets or clotting factors are not necessary for LVP.

TIPS

A TIPS is a prosthesis that bridges a branch of the portal vein with a branch of the hepatic vein and is very effective in reducing the portal pressure. Physiologically, the lowering of portal pressure allows the gradual return of the splanchnic volume to the central circulation, thereby slowly filling the EABV. This is associated with the gradual suppression of the activated renin-angiotensin-aldosterone and the sympathetic nervous systems, accompanied by a gradual reduction in the severity of renal sodium retention in these patients. When the activities of these neurohormonal systems have fallen to below their sodium retaining thresholds, we can expect ascites clearance. This usually takes about 3 to 6 months. Six months post TIPS placement in patients with RA, 45% of patients show complete response, whilst 63% show partial response. Eventually, TIPS is effective in controlling ascites in approximately 80% of patients. Therefore, it is important to manage patient expectation, as TIPS does not clear the ascites instantly; rather, the ascites will gradually diminish until it eventually disappears. While the ascites is still present, it is important to continue dietary sodium restriction until total ascites clearance. The use of diuretics post-TIPS is controversial, as this tends to reduce the EABV, and theoretically can delay the clearance of ascites.

Several randomized controlled trials have compared TIPS vs. LVP in the management of RA (Table 2), and all shown that TIPS is significantly better than LVP in the control of ascites. However, the survival advantage of TIPS over LVP in patients with RA was not established until recently, and this is dependent on careful patient selection. In general, younger patients who have a low Model of End-stage Liver Disease (MELD) score tend to do well with excellent transplant-free survival at 3 years. This is especially true if the patient’s major problem is related to portal hypertension and not liver dysfunction. A small increase in patient’s age, MELD score, or hemodynamic parameters can decrease transplant-
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<th>Study</th>
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<td>Lebrec et al.15 (1996)</td>
<td>25/25</td>
<td>Cirrhosis &amp; RA defined as: no response to maximum diuretics for 5 days while in hospital, or ≥2 admissions for tense ascites in ≤4 months</td>
<td>Age &gt;70 years&lt;br&gt;HE ≥ grade 2&lt;br&gt;PVT&lt;br&gt;Biliary obstruction&lt;br&gt;sCr &gt;1.7 mg/dL&lt;br&gt;HCC&lt;br&gt;Active bacterial infection&lt;br&gt;Severe extra-hepatic disease&lt;br&gt;Pulmonary hypertension</td>
<td>1) HE: TIPS group, severe recurrent grade 3 HE in 2 patients, mild HE in 1 patient; LVP group, no HE&lt;br&gt;2) Survival at 2 years: TIPS group, 29%±13%; paracentesis group, 60%±16%</td>
<td>Minimum or no ascites at 4 months of all surviving patients: TIPS group, 5/6; paracentesis group, 1/10</td>
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<td>Rössle et al.16 (2000)</td>
<td>150/60</td>
<td>Cirrhosis &amp; RA as defined by IAC criteria*: 55%&lt;br&gt;Cirrhosis &amp; recurrent ascites: 45%</td>
<td>HE ≥ grade 2&lt;br&gt;PVT&lt;br&gt;Bilirubin &gt;5 mg/dL&lt;br&gt;sCr &gt;3.0 mg/dL&lt;br&gt;Advanced HCC&lt;br&gt;Hepatic hydrothorax&lt;br&gt;Technical failure of paracentesis</td>
<td>1) HE at mean follow-up of approximately 44 months: TIPS group, 58%; LVP group, 48%&lt;br&gt;2) Survival at 2 years: TIPS group, 58%; paracentesis group, 32% (P=0.11)</td>
<td>Complete response at 6 months: TIPS group, 79%; paracentesis group, 24% (P=0.001)&lt;br&gt;Partial response at 6 months: TIPS group, 9%; paracentesis group, 19% (P=N.S.)</td>
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<td>Ginès et al.17 (2002)</td>
<td>119/70</td>
<td>Cirrhosis &amp; RA as defined by IAC criteria*</td>
<td>Age &lt;18 or &gt;75 years&lt;br&gt;HE ≥ grade 2&lt;br&gt;PVT&lt;br&gt;Bilirubin &gt;10 mg/dL&lt;br&gt;sCr &gt;3.0 mg/dL&lt;br&gt;Prothrombin index &lt;40%&lt;br&gt;Platelet &lt;40×10⁹/L&lt;br&gt;HCC&lt;br&gt;CHF&lt;br&gt;Parenchymal renal disease</td>
<td>1) Severe HE at mean follow-up of 282–325 days: TIPS group, 60%; LVP group, 34% (P=0.03)&lt;br&gt;2) Transplant free survival at 2 years: TIPS group, 26%; paracentesis group, 30% (P=0.51)</td>
<td>Median time to ascites recurrence: TIPS group, 171 days; paracentesis group, 20 days (P&lt;0.0001)</td>
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<td>Sanyal et al. (2003)</td>
<td>525/109</td>
<td>Cirrhosis &amp; RA as defined by IAC criteria*</td>
<td>HE ≥ grade 2</td>
<td>1) Severe HE: TIPS group, 38%; LVP group, 21% (P = 0.058)</td>
<td>Recurrent ascites at 180 days: TIPS group, 6/57 (10.5%); paracentesis group, 26/52 (50%) (P &lt; 0.0001)</td>
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<td>Bilirubin &gt; 10 mg/dL</td>
<td>2) Transplant free survival: TIPS group, 19.6 months; paracentesis group, 12.4 months (P = 0.77)</td>
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<td>Salerno et al. (2007)</td>
<td>137/66</td>
<td>Cirrhosis &amp; RA as defined by IAC criteria*: 68% and Cirrhosis &amp; recidivant ascites: 32%</td>
<td>Age &gt; 72 years</td>
<td>1) HE: TIPS group, 61%; LVP group, 39% (P = 0.086)</td>
<td>Failure of treatment: TIPS group, 7/33 (21.2%); paracentesis group, 19/33 (57.6%) (P = 0.0012)</td>
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<td>HE ≥ grade 2</td>
<td>2) Transplant free survival at 2 years: TIPS group, 59%; paracentesis group, 29% (P = 0.021)</td>
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<td>Narahara et al. (2011)</td>
<td>78/60</td>
<td>Cirrhosis &amp; RA as defined by IAC criteria*</td>
<td>Age &gt; 70 years</td>
<td>1) Severe HE: TIPS group, 67%; LVP group, 17% (P &lt; 0.01)</td>
<td>Complete or partial response at 1 year: TIPS group, 20/30 (67%); paracentesis group, 8/30 (17%) (P &lt; 0.005)</td>
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<td>sCr &lt; 1.9 mg/dL</td>
<td>Episodes of HE</td>
<td>2) Overall survival at 2 years: TIPS group, 64%; paracentesis group, 35% (P &lt; 0.005)</td>
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TIPS, transjugular intrahepatic portosystemic shunt; RA, refractory ascites; HE, hepatic encephalopathy; PVT, portal vein thrombosis; sCr, serum creatinine; HCC, hepatocellular carcinoma; LVP, large volume paracentesis; IAC, International Ascites Club; N.S., not significant; CHF, congestive heart failure; INR, international normalized ratio; Gl, gastrointestinal; PV, portal vein.

*Diagnosis of refractory ascites by the International Ascites Club: Salerno et al. or Table 1.
free survival significantly.\textsuperscript{50} For the former patient, TIPS can be used as a definitive treatment for the RA, while for the latter patient, TIPS is used as a bridge therapy while waiting for a liver transplant. A recent study suggests that a TIPS inserted in patients with recurrent ascites (the need for at least three LVPs within 12 months with a time interval of >4 weeks between LVPs) could result in fewer side effects and improved survival when compared to LVP (93\% vs. 52\%, \textit{P}=0.003);\textsuperscript{51} in particular, the post-TIPS incidence of hepatic encephalopathy (HE) (see below) was similar between the two groups. However, this study has not been replicated, and therefore, TIPS insertion at the stage of recurrent ascites cannot be recommended as standard of care yet.

The insertion of TIPS is associated with many complications. Immediate complications related to the procedure include arrhythmia, hemoperitoneum, and liver capsule rupture, which in experienced hands are rare. Other complications in the early post-TIPS period include shunt migration, kinking, and ischemic hepatitis as evidenced by a significant rise in liver enzymes and hemolytic anemia. Therefore, patients may remain jaundiced for several weeks to months post TIPS. When bare stents were used in earlier times, shunt stenosis occurred frequently. These are now relatively uncommon with the polytetrafluoroethylene (PTFE) covered stent,\textsuperscript{52} related to reduction in the thickness of the neointima. The major clinical complication is HE, either newly onset or worsening of existing HE irrespectively of the type of stent used, estimated to occur in 30–50\% of patients.\textsuperscript{53,54} The risk factors for the development of HE includes advancing age, higher Child-Pugh and MELD scores, prior episodes of spontaneous HE, sarcopenia and lower portal systemic pressure gradient post-TIPS.\textsuperscript{55} The latter is usually associated with a maximally dilated TIPS. A recent report confirmed a lower incidence of HE (27\%) in patients who had their PTFE stent deliberately under-dilated to 6 mm compared to patients whose stent was dilated to 8–10 mm (54\%) without any negative impact on variceal bleeding or ascites recurrence or on the incidence of stent thrombosis.\textsuperscript{56} Therefore, it appears that either under-dilation or smaller diameter stents are more appropriate to reduce the likelihood of post-TIPS HE. The use of lactulose and rifaximin pre-emptively has also been shown to provide better HE control in the post-TIPS period.\textsuperscript{57,58} Another potential complication of TIPS insertion is the development of cardiac failure post-TIPS. The placement of TIPS returns a significant volume from the splanchnic circulation to the systemic circulation, and the cardiac output can increase by up to 50\%.\textsuperscript{59} Therefore, patients with pre-existing cardiac dysfunction, whether systolic incompetence or abnormal diastolic relaxation, or the presence of pulmonary hypertension are at risk for post-TIPS cardiac decompensation. The appropriate pre-TIPS cardiac investigations include electrocardiogram, echocardiogram, and measurement of brain natriuretic peptide (BNP).\textsuperscript{59} A normal cardiac investigation with a BNP level of <40 pg/mL and a pro-N-terminal BNP of <125 pg/mL have been reported as indicators that will rule out cardiac decompensation post-TIPS.\textsuperscript{60}

Other pre-TIPS investigations include assessing for sites of infection, especially biliary and dental infections. Once the TIPS is inserted, any source of infection that can reach the TIPS via the blood stream may produce endotipsitis. Although this is a rare complication, the occurrence of endotipsitis will lead to recurrence of bacteremia which may not be eradicated even with prolonged courses of antibiotics.\textsuperscript{61}

Appropriate patient selection is very important to optimize patient response to TIPS with clearance of ascites and to reduce the likelihood for complications. Various academic societies have recommended against placing a TIPS in patients who are older than 70 years of age, with a MELD score of >18, who have had spontaneous HE \textgtr= grade 2, or the presence of cardiac failure, pulmonary hypertension, liver cancer, sepsis, or occlusive portal vein thrombosis.\textsuperscript{11,62} Clearance of ascites with TIPS is associated with improved quality of life,\textsuperscript{63} better nitrogen balance,\textsuperscript{64} significant muscle gain,\textsuperscript{65} and improved survival.\textsuperscript{48,66,67} A recently validated Freiburg Index of Post-TIPS survival (FIPS) included age, bilirubin, albumin, and creatinine in its prediction of high risk for mortality post-TIPS. Patients in the high-risk category had a median post-TIPS survival of 5 months vs. 48 months in the low-risk group (\textit{P}<0.001).\textsuperscript{68} However, the predictive power of FIPS is not as accurate in patients who have received an early TIPS for ascites that has not yet reached the refractory stage. A further study showed improved post-TIPS survival in patients who received an 8 mm covered stent compared to those who received a 10 mm covered stent.\textsuperscript{69}

**The automated low flow ascites pump (alfapump)**

The alfapump is a programmable and rechargeable device that is implanted subcutaneously. It slowly pumps the ascites...
from the peritoneal cavity via a peritoneal catheter and discharges it via a bladder catheter into the bladder, from there it is discharged as urine (Fig. 4). Effectively, it is performing continuous small volume paracentesis. The device is programmed to pump ascites for up to 16 hours during awake hours, so not to disturb the patient’s sleep by requiring the patient to urinate the ascites at night. The device is fitted with various sensors in the peritoneum and in the bladder so that it will stop pumping if there is little or no ascites. The rate of ascites discharge can also be adjusted according to the patient’s dietary sodium consumption. Therefore, the management of ascites is individualized. Usually, the use of the alfapump system does not require the concomitant use of albumin infusions.

A randomized controlled trial, several prospective, and retrospective studies as well as a meta-analysis have shown that the alfapump is effective in the control of ascites by reducing the frequency and volume of paracenteses. The initial study showed a high incidence of complications including infection of the alfapump system, pump malfunction, and dislodgement of catheters. With the use of prophylactic antibiotics, refinement of pump design, and the implantation techniques, these complications have become less frequent. A proportion of patients still develop renal dysfunction despite the slow continuous discharge of ascites. A physiological study showed that there is still activation of the various vasoconstrictor systems with the small volume but continuous paracentesis. It has been suggested that patients should be monitored for the development of renal dysfunction, and given intermittent albumin as required. Therefore, it is prudent to avoid alfapump insertion in patients with renal dysfunction with serum creatinine >132 μmol/L (1.5 mg/dL) or estimated glomerular filtration rate <30 mL/min/1.32 m². Other contra-indications for alfapump insertion include at least 2 or more systemic or local abdominal infections in the previous 6 months, recent intra-abdominal surgery, history of bladder cancer, previous solid organ transplantation, and bilirubin level >85 μmol/L.

Once the ascites is under control, patients show significant improvement in their mobility and quality of life. The effects of the alfapump on the survival of these patients has not been formally studied but has been shown to be at least the same as patients who undergo regular LVP.

**Liver transplantation**

Liver transplantation remains the definitive treatment for patients with RA and concomitant liver dysfunction. However, for patients whose major complications of liver cirrhosis are related to portal hypertension alone without significant liver dysfunction, their priority for liver transplantation remains low. A recent publication has shown that patients with
ascites and a MELD score of <15 still had a mortality risk of 47.5% at 1 year without a liver transplant, related to infectious causes. The presence of persistent ascites is equivalent to adding 4.5 MELD or 3.5 MELD-Na score points to the patient’s calculated MELD, especially in patients with a lower calculated MELD score of less than 21. Therefore, patients with RA as the only manifestation of cirrhosis should still be considered for liver transplantation despite fairly low MELD score. Patients with RA and concomitant hyponatremia will have higher priority for liver transplant as their ranking will be captured by a higher MELD-Na score. After liver transplantation, ascites may persist for weeks to months, as it takes time for the systemic and renal hemodynamics to adjust back to normal, especially if high portal inflow persists after liver transplantation. Therefore, patients are advised to remain on sodium-restricted diet in the post-transplant period until ascites disappears.

**Treatment of RA in patients with chronic kidney disease (CKD)**

CKD has always been known to be associated with RA. It used to be known as type 2 hepatorenal syndrome. However, the prevalence of CKD in cirrhosis is increasing, related to the increased prevalence of nonalcoholic steatohepatitis and its associated conditions such as diabetes mellitus and systemic hypertension. Furthermore, it is now also recognized that CKD can develop after repeat episodes of acute kidney injury. There is very little published literature specifically on the management of ascites in patients with CKD. In general, patients with RA and CKD should have their ascites managed the same way as patients without CKD. However, the volume of ascites removed at paracentesis should not be excessive, as the risk for acute kidney injury post-paracentesis is proportional to the volume of ascites removed. The insertion of a TIPS to treat RA in patients with CKD also appears to be safe. When these patients are being evaluated for liver transplantation, consideration should be given for combined liver kidney transplant, especially in patients with stage ≥3b CKD with their GFR at ≤44 mL/min/1.73 m² for more than 3 months.

**Palliative care in patients with RA**

There remains a significant number of patients with RA who are not liver transplant candidates. They are not appropriate as TIPS recipients because of comorbid conditions, and the alfapump system is not widely available. Therefore, LVP remains the only option available to these patients as a treatment for their RA. Recently, there has been a push for these patients to receive palliative care as their survival is rather limited. There is some interest in the use of a tunneled catheter to provide long-term ascites drainage at home rather than having regular hospital visits for LVPs. Some patients reported preference for the tunneled catheter as this avoids repeat LVPs in hospital, and therefore improved quality of life. However, bacterial peritonitis, ascites leakage, and local cellulitis remain concerns. Therefore, until there are well-designed randomized controlled trials to confirm its safety and efficacy, this cannot be recommended as standard of care for patients with advanced liver disease and RA.

**CONCLUSION**

RA represents further deterioration of the patient with ascites when the ascites is no longer responsive to diuretic therapy. Despite this, sodium restriction remains an integral part of the management of these patients. LVP remains the cornerstone of ascites management, but care needs to be taken to avoid inducing the development of PPCD. Regular infusions of albumin may be of benefits but remain to be proven. In the appropriate patients, TIPS insertion can provide permanent relief of ascites. The use of an alfapump system in patients who are not TIPS candidates can provide slow and continuous ascites removal, therefore eliminating abdominal bloating with associated benefits of increased appetite, and eventual improved mobility. This requires long-term use of antibiotics as prophylaxis against infection of the alfapump system. As the prognosis of patients with cirrhosis is negatively impacted by the presence of RA, these patients need to be assessed for liver transplant. In patients with RA and CKD, consideration should be given for combined liver-kidney transplant.

**Conflicts of Interest**

Sequana Medical: Consultant, and grant support to institution.
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Wnt signaling in liver regeneration, disease, and cancer

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INTRODUCTION

Liver regeneration has been extensively studied.1-3 In vivo studies have shown that partial hepatectomy or chemical injury activates extracellular and intracellular signaling pathways, leading to liver regeneration. Hepatocyte loss during chronic liver diseases triggers compensatory proliferation of the surviving hepatocytes.4-6 Apart from liver regeneration in physiological conditions, genotoxic risk factors might lead them to convert to neoplasia. Hepatitis virus, alcohol abuse, non-alcoholic fatty liver disease (NAFLD), and aflatoxin-B1 exposure are also the main etiological factors to induce the development of precancerous lesions in the liver. Liver cancer is one of the top 10 lethal cancers worldwide. Its estimated death rate in 2021 is 6% in males and 4% in females.7 Liver cancer consists of hepatocellular carcinoma (HCC), cholan-
giocarcinoma (CCA), hepatoblastoma (HB), and several other rare tumors (angiosarcoma, intraductal papillary neoplasm of the bile duct, and mucinous cystic neoplasm). HCC is the most common primary liver cancer frequently developed with chronic liver disease, such as cirrhosis caused by hepatitis virus infection.8

Among various signaling pathways associated with liver biology,9-12 Wnt signaling is involved in all stages of liver disease progression, from liver injury to inflammation, fibrosis, cirrhosis, and tumorigenesis. Several Wnt ligands are secreted by various hepatic cells, including hepatocytes, stellate cells, Kupffer cells, biliary epithelial cells, and sinusoidal endothelial cells.13-16 Based on the oncogenic roles of Wnt signaling in cancer, several components and regulators of Wnt signaling have been proposed as the druggable targets to improve the current therapeutic efficacy in the liver cancer treatment.17

Herein, we review the roles of Wnt signaling in liver regeneration and liver tumorigenesis and the therapeutic targets of Wnt signaling in liver cancer treatment.

**Wnt SIGNALING**

Wnt signaling is evolutionarily conserved and orchestrates various cellular processes, including cell proliferation, differentiation, migration, polarity, stemness, and lineage plasticity.18,19 Consequently, Wnt signaling plays a pivotal role in organogenesis, tissue homeostasis, tissue regeneration, and tumorigenesis.20-25 The Wnt signaling is triggered by the binding of the Wnt ligands to the frizzed (FZD) receptors. The mammals have 19 Wnt ligands and 10 FZD receptors,26 resulting in the complexity and specificity in Wnt signaling activation. Based on the involvement of β-catenin, a key component of Wnt signaling, Wnt signaling is generally classified into canonical (β-catenin-mediated) and non-canonical (β-catenin-independent) Wnt signaling (Fig. 1). In the canonical Wnt/β-catenin pathway, the protein destruction complex (casein kinase 1 [CK1], glycogen synthase kinase 3 [GSK3], adenomatous polyposis coli [APC], and axis inhibition proteins [AXINs]) targets the β-catenin protein for degradation via CK1 and GSK3-mediated sequential phosphorylation at the N-terminus (Ser-45, Thr-41, Ser-37, and Ser-33) of β-catenin followed by β-TrCP, an E3 ligase, recruitment. Conversely, binding of the canonical Wnt ligands to the FZD receptors and LRPS/6 co-receptors activates dishevelled (DVL), which inhibits the protein destruction complex. As a result, β-catenin protein is stabilized and translocated into the nucleus to transactivate the canonical Wnt target genes by replacing the co-repressors associated with the T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) with the co-activators. Non-canonical Wnt signaling pathways include the planar cell polarity pathway (involved in c-Jun N-terminal kinase [JNK] activation, small GTPase activation, and cytoskeletal rearrangement), and the Wnt/β-catenin pathways (activating phospholipase C [PLC] and protein kinase C [PKC]).18,19

**Wnt SIGNALING IN LIVER REGENERATION**

Upon partial hepatectomy or acute liver injury, the number of hepatocytes is drastically reduced. Various signaling pathways (epidermal growth factor [EGF], hepatocyte growth factor [HGF], Wnt/β-catenin, and Notch) stimulate the hepatocytes in the G0 phase to proliferate, compensating tissue loss and restoring the physiological functions of the liver.27,29 During liver regeneration, endothelial cells under shear stress produce Wnts to activate Wnt/β-catenin signaling in hepatocytes. Additionally, the organ precisely senses the size of the

**Abbreviations:**

AFB1, aflatoxin type B1; ALD, alcoholic liver disease; APC, adenomatous polyposis coli; AXIN, axis inhibition protein; CCA, cholangiocarcinoma; CCL, carbon tetrachloride; CEBPA, CCAAT enhancer-binding protein α; CK1, casein kinase 1; CREBBP/CBP, CREB binding protein; CV, central vein; DDC, 1,4-dihydro-2,4,6-trimethyl-pyridine-3,5-dicarboxylate; DENT, diethylthiourease; DKK1, Dickkopf 1; DVL, dishevelled; E2F1, E2F transcription factor 1; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; ETOH, ethanol; FZD, frizzled; GPC3, glypican-3; GSK3, glycogen synthase kinase 3; HB, hepatoblastoma; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HGF, hepatocyte growth factor; Ig, immunoglobulin; IGF1, insulin-like growth factor 1; JNK, c-Jun N-terminal kinase; KC, knock-out; LGR5, leucine-containing repeat G-protein-coupled receptor 5; LOF, loss-of-function; LRPS, lipoprotein receptor-related protein 6; MAPK, mitogen-activated protein kinase; MCF, monochrome chemoattractant protein; MMP7, matrix metalloproteinase; mTOR, mammalian target of rapamycin; MIRs, multisecular bodies; NAFLD, non-alcoholic fatty liver disease; NCOA2, nuclear receptor coactivator 2; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; PEG-IFN, pegylated-Interferon-α2a; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PORCN, porcupine; PPARG, peroxisome proliferator-activated receptor γ; RanBP3, Ran-binding protein 3; ROS, reactive oxygen species; SAL, salinomycin; SFRP1, secreted frizzled-related protein 1; sFZD7, secreted FZD7; SREBF, sterol regulatory element binding transcription factor; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor; TMEM99, transmembrane protein 9; TNKS, tankyrase; VEGF, vascular endothelial growth factor; Wnt, Wnt inhibitory factor 1; WISP1, Wnt1-inducible-signaling pathway protein 1

34

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Several animal models (rat, mouse, and zebrafish) were utilized for liver regeneration study. The partial hepatectomy is the classic strategy to create the murine liver regeneration model. Carbon tetrachloride (CCl4) is a frequently used chemical to induce liver injury in rats and mice. Meanwhile, several dietary-induced liver injury models are also commonly used. Biliary injury and regeneration can be induced by the 1,4-dihydro-2,4,6-trimethyl-pyridine-3,5-dicarboxylate (DCC) diet. Besides murine models, zebrafish emerged as a potent model for drug screening of liver generation. Partial hepatectomy, drug-induced liver injury, and nitroreductase-mediated hepatocyte ablation were employed to establish the zebrafish liver injury model. The transient activation of the canonical Wnt signaling is indispensable for liver regeneration (Fig. 2). In rat models, overexpressed Wnt1 and nuclear β-catenin are predominantly accumulated in remaining parenchymal cells after 70% partial hepatectomy. The level of β-catenin increased within 5 minutes after hepatectomy, accompanied by its nuclear translocation and subsequent target gene expression for hepatocyte proliferation. Significantly, genetic ablation of β-catenin/Ctnnb1 impairs liver regeneration of mice from partial hepatectomy. The liver-specific Ctnnb1 knock-out (KO) delayed DNA synthesis and hepatocyte proliferation in mice after partial hepatectomy. Conversely, activation of
Wnt/β-catenin signaling accelerates liver regeneration in the zebrafish model. It was also shown that liver damage upregulated leucine-containing repeat G-protein-coupled receptor 5 (LGR5) and AXIN2 in the hepatocytes. LGR5 is a marker of actively dividing stem and progenitor cells in Wnt-driven self-renewing tissues. LGR5 interacts with FZD and lipoprotein receptor-related protein 6 (LRP6) to enhance phosphorylation of LRP6, which in turn enhances the Wnt/β-catenin signaling. While Lgr5 is not expressed in healthy adult livers, after liver damage, Lgr5+ cells appear near the bile ducts, consistent with strong activation of the Wnt signaling. AXIN2 is another Wnt downstream target gene transactivated by β-catenin. Like AXIN1, AXIN2 combined with other destruction complex components degrades β-catenin, serving as a negative feedback regulator of the Wnt signaling.

Other than core components of Wnt signaling, additional regulators of Wnt signaling were implicated in liver regeneration. Recently, our group identified the transmembrane protein 9 (TMEM9) gene as an amplifier of Wnt/β-catenin signaling. TMEM9 is a type I transmembrane protein primarily localized in lysosomes and multivesicular bodies (MVBs). While the ablation of TMEM9 inhibits the activity of the Wnt/β-catenin signaling, β-catenin transactivates TMEM9, leading to hyperactivation of Wnt/β-catenin signaling. Interestingly, TMEM9 is highly expressed in hepatocytes around the central vein (CV) of regenerating liver. TMEM9 hyperactivates Wnt/β-catenin signaling to promote liver regeneration through lysosomal degradation of APC protein. Tmem9 KO impairs CCl4-induced liver regeneration with downregulation of Wnt/β-catenin signaling.

In addition to the role of Wnt/β-catenin signaling in regeneration, sustained activation of the Wnt signaling is associated with the progression of chronic liver diseases and liver tumorigenesis (Fig. 2). Additionally, reactive oxygen species (ROS) and lipid peroxide are the risk factors for the development of the precancerous lesion in the liver. However, the crosstalk between Wnt signaling and ROS has not been fully revealed in the liver. It was reported that β-catenin can be further stabilized by ROS. Meanwhile, lipid peroxidation products mainly generated by ROS activate the canonical Wnt pathway through oxidative stress. Therefore, it is likely the potential crosstalk between Wnt signaling and ROS might contribute to liver cancer development.

Accumulating evidence suggests that many chronic liver diseases contribute to liver cancer development, described below.

**Wnt SIGNALING IN PRECANCEROUS LIVER LESION**

**Hepatitis virus**

Globally distributed hepatitis B virus (HBV) and hepatitis C virus (HCV) are the crucial triggers of HCC initiation. Both HBV
and HCV can induce chronic infections and are essential pathogenic factors in cirrhosis and liver cancer (Fig. 3). The epidemiological data show that more than 70% of patients with liver cancer have HBV infection, 10–20% have HCV infection, and a significant proportion of patients have both HBV and HCV infection.

After infection, the DNA of HBV is integrated into the host genome, inducing genomic instability and transactivation of cancer-related genes, which culminates in the formation of early cancer cell clones. Mechanistically, HBV contributes to HCC development through direct and indirect means. Direct mechanisms include virus mutations, HBV DNA integration, growth regulatory genes activation by HBV-encoded proteins. Indirect mechanisms include the activation of cellular oncogenes associated with HBV DNA integration, genetic instability induced by viral integration or the regulatory protein HBx, and the development of liver disease mediated by immune enhancement due to viral proteins.

Both hepatitis B virus surface antigen (HBsAg) and HBx modulate the expressions of genes involved in Wnt signaling activation. HBsAg activates the transcription factor LEF1 of the Wnt signaling. The X protein encoded by the hepatitis B virus has a vital role in stimulating viral gene expression and replication, critical for maintaining chronic carrier status. HBx, a 17 kDa multifunctional protein, upregulates the expression of Wnt ligands (WNT1 and WNT3), the receptor (FZD2 and FZD7), a component of the destruction complex (GSK3β), E-cadherin, and Wnt1-inducible-signaling pathway protein 1 (WISP1), a suppressor of Wnt antagonists (secreted frizzled-related protein 1 [SFRP1] and SFRP5). On the other hand, the Wnt signaling key components (β-catenin and AXIN1) are highly mutated in HBV-associated HCC. Loss-of-function (LOF) mutations of the AXIN1 are observed in HBV-HCC patients. In HBV and/or HCV-associated HCC patients, the most frequent mutation in the CTNNB1 gene is enriched in the exon 3 encoding the N-terminal phosphorylation sites.

These aberrantly controlled genes in Wnt signaling subsequently promote and lead to the development of HCC.

The oncogenic mechanism of HCV in liver cancer is mainly mediated by Wnt/β-catenin signaling hyperactivation via the core protein and two nonstructural proteins, NS3 and NSSA. The core protein (HCV core antigen) is a significant component of HCV. It regulates hepatocyte transcription and promotes Wnt/β-catenin signaling by upregulating Wnt ligands (WNT1 and WNT3A), FZD receptors, and LRP5/6. Additionally, at the early stage of HCV infection, the secreted Wnt antagonists, SFRP2 and Dickkopf 1 (DKK1), are downregulated by their promoter hypermethylation. HCV core protein also promotes hypermethylation of the CDH1 gene promoter region, destabilizing the cadherin-catenin-actin complex for β-catenin release and activation. HCV core protein also promotes liver fibrosis by enhancing the activation and survival of hepatic stellate cells.
Alcohol abuse

Alcohol is a well-known risk factor for liver cancer. Alcoholic liver disease (ALD) is a chronic liver disease caused by long-term alcohol consumption (Fig. 3). ALD is characterized by the fatty liver at the beginning, then progressed to alcoholic hepatitis, liver fibrosis, and cirrhosis, which is pathologically associated with the precancerous lesions of HCC. In vivo, ethanol (EtOH) is metabolized into the reactive metabolite acetaldehyde, promoting liver tumorigenesis. Mice administered with the chemical carcinogen, diethylnitrosamine (DEN), for 7 weeks and the subsequent EtOH feeding for 16 weeks exhibited the increased total number of cancer foci and liver tumors.25 Also, these tumors showed a 3- to 4-fold increase in the expression of proliferation markers and an increased expression of β-catenin, compared to non-tumor hepatocytes.25 In a rat model of chronic liver disease, EtOH-treated liver was accompanied by the increased proliferation of hepatocytes, depletion of retinol and retinoic acid storage, augmented expression of phospho-GSK3β at the cell membrane, significant upregulation of soluble Wnt ligands (Wnt2 and Wnt7a), accumulation of nuclear β-catenin, and upregulation of β-catenin target genes (cyclin D1/CCND1, c-Myc/MYC, WISP1, and matrix metalloproteinase [MMP7]). These data suggest that long-term EtOH consumption activates the Wnt/β-catenin signaling and increases hepatocyte proliferation, promoting liver tumorigenesis.26 Additionally, ROS accumulation, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein (MCP)-1 upregulation, and activation of extracellular signal-regulated kinase (ERK)-mitogen-activated protein kinase (MAPK) signaling also contribute to EtOH-induced liver tumorigenesis.27-30

NAFLD

The increasing prevalence of NAFLD was caused by an over-nourished lifestyle.31,32 NAFLD is characterized by fat accumulation in the liver, evolving to end-stage liver diseases such as cirrhosis and HCC (Fig. 3).33 The main risk factors of NAFLD include central obesity, overnutrition, insulin resistance, and metabolic syndrome.34 In severe NAFLD, many tissue repair-related genes (TMEM204, FGFR2, matrix molecules, and matrix remodeling factors) were hypomethylated at their promoters and overexpressed. Conversely, genes in specific metabolic pathways (lipid metabolism, cytochrome P450 family, multidrug resistance, and fatty acid anabolic pathways) were hypermethylated and silenced.35 Hyperinsulinemia is one of the risk factors of NAFLD.36 SOX17 plays a vital role in regulating insulin secretion. Sox17 KO mice display high susceptibility to high-fat diet-induced hyperglycemia and diabetes.37 SOX17 directly interacts with the TCF/LEF transcription factor to repress the transcription of Wnt signaling target genes. The methylation of the SOX17 promoter is a frequent event in human cancers. Epigenetic silencing of SOX17 contributes to the aberrant activation of Wnt/β-catenin signaling,38 accelerating progression from NAFLD to HCC. Besides, β-catenin inhibits the expression of CCAAT enhancer-binding protein α (CEBPA) and peroxisome proliferator-activated receptor γ (PPARG), which in turn inhibits the preadipocyte differentiation.39 As the co-receptor of the Wnt/β-catenin signaling, LRP6 induces lipid accumulation in the liver via insulin-like growth factor 1 (IGF1)/AKT/mammalian target of rapamycin (mTOR)/sterol regulatory element binding transcription factor (SREBF) 1/2 signaling. Intriguingly, inhibiting the non-canonical Wnt signaling reduces lipid accumulation and inflammation.40 Therefore, while reducing the effects of NAFLD risk factors, inhibition of the Wnt signaling is also essential for attenuating the development of NAFLD and preventing the initiation of HCC.

Aflatoxin-B1 exposure

Among the aflatoxins, aflatoxin type B1 (AFB1) primarily targets the liver as a highly potent hepatotoxin and hepatocarcinogen (Fig. 3). AFB1 impairs DNA repair processes, resulting in severe DNA mutagenesis, and also inhibits DNA and RNA metabolism. This pathological event ultimately leads to excessive liver lipid accumulation, liver enlargement, bile duct epithelial hyperplasia, and liver cancer. The potency of aflatoxin to cause liver cancer is significantly enhanced in the presence of HBV infection. Under chronic HBV infection, cytochrome P450s could metabolize inactive AFB1 to mutagenic AFB1-8,9-epoxide. Also, the infection leads to hepatocyte necrosis and regeneration, producing oxygen and nitrogen reactive species and increasing the incidence of AFB1-induced mutagenesis.41 Clinical studies have shown that CTNNB1 mutations are present in approximately one-quarter of HCC in areas with low aflatoxin B1 exposure. Interestingly,
these CTNNB1 mutations were similar to those previously reported in the human HCC.92

**Wnt SIGNALING IN LIVER CANCER**

**HCC**

HCC is a common and fatal malignancy worldwide.93 Regardless of the risk factors mentioned above, aberrant hyperactivation of Wnt/β-catenin signaling is observed in 95% of HCCs.94 The most common genetic mutations of the Wnt signaling in HCC are the gain-of-function mutations in the CTNNB1 gene encoding β-catenin,61,95 which is somewhat distinct from colorectal cancer where Wnt/β-catenin signaling hyperactivation is mainly driven by the APC gene inactivation.96 Missense mutations of CTNNB1 exon 3 were observed in 18.1% of HCC cases. Missense mutations at codons 32, 33, 38, or 45 of the CTNNB1 gene lead to the unphosphorylation of the N-terminus of β-catenin for its stabilization, nuclear translocation, and target gene transactivation.60 Secondly, the LOF mutations in the AXIN1 gene were observed in 5–19% of HCC cases.97 CTNNB1 and AXIN1 mutations occur in patients with advanced HCC (Fig. 3).98-100 Importantly, hyperactivation of the Wnt signaling is considered a hallmark of advanced HCC.101 It should also be noted that mutations in the CTNNB1 and AXIN1 genes lead to different HCC subtypes accompanied by distinct clinical and pathological features. CTNNB1 mutations are associated with less aggressive HCC, including chromosomally stable and highly differentiated tumors,102 with a better prognosis.103 In contrast, AXIN1 mutations occur more frequently in more aggressive HCC tumors characterized by hypodifferentiated tumor cells and chromatin instability.104 Consistently, the HCC tumors with CTNNB1 mutations or AXIN1 mutations showed different target gene expression.61,95,103

**CCA**

CCA is ranked as the second most common hepatobiliary cancer after HCC. CCA originates mainly from differentiated bile duct epithelial cells.104 CCA is often diagnosed at an advanced stage with a poor prognosis. Current chemotherapy has not improved the survival rate of unresectable CCA patients. Clinical and preclinical studies have shown that activation of the Wnt/β-catenin signaling occurs throughout the initiation and progression of CCA. Wnt ligands (WNT2, WNT7b, and WNT10A) and TCF4 are upregulated in CCA, accompanied by nuclear translocation of β-catenin.105,106 The progression of epithelial-mesenchymal transition (EMT) was observed in CCA, represented by the disrupted epithelial cell-cell junctions and mesenchymal characteristics.107-109 Wnt/β-catenin signaling is one of the critical pathways promoting the EMT transition.110,111 In CCA cells, suppression of Wnt/β-catenin signaling increased E-cadherin and downregulated vimentin,112,113 suggesting that the Wnt/β-catenin signaling is associated with EMT during CCA tumorigenesis. β-catenin interacts with E-cadherin to form the cadherin-catenin-actin complex, maintaining epithelial cell adhesion, cytoskeleton, and integrity. During CCA development, the decreased E-cadherin releases β-catenin, resulting in β-catenin accumulation and nuclear translocation.111 Then, β-catenin activates the transcription of twist, snails, and ZEB1 to induce the EMT process in CCA cells.111

**HB**

HB is a rare malignant tumor found in infants and children.114 The preclinical and clinical studies showed the hyperactivation of Wnt/β-catenin signaling in HB. In HB cases, β-catenin was found to be increased in the cytoplasm and nucleus of the tumor cells.115,116 While CTNNB1 mutations are limited in the exon 3 in embryonal HB, the CTNNB1 mutations in fetal HB encompass exon 3 and 4.117 Meanwhile, missense, deletion, or insertion mutations in the AXIN1 gene were detected in 8% of HB cases.118

**MANIPULATING Wnt SIGNALING**

**Porcupine (PORCN)**

PORCN is a membranous protein mainly localized in the endoplasmic reticulum. PORCN mediates the palmitoylation of Wnt ligands, an essential process for Wnt ligands secretion and ligand-frizzled receptor binding.119-121 Genetic and pharmacological blockade of PORCN reduces palmitoylation and inhibits the secretion of Wnt ligands, suppressing Wnt signaling.122 The clinical trials showed promising results of PORCN inhibitors in HCC treatment. ETC159, CGX1321, and RXC004
have entered phase I clinical trials, and IWP12 is still in the preclinical studies (Fig. 4). In mouse models, Porcn KO induces embryonic lethality. Porcn inhibition could cause adverse effects on bone homeostasis.

**Wnt ligands**

In physiological conditions, Wnt signaling is activated by binding of secreted Wnt ligands to LRPS/6 coreceptors and FZD receptors. Thus, targeting Wnt ligands by chemicals or neutralizing antibodies efficiently inhibits Wnt signaling. Based on the high expression of WNT1 in human HCC cell lines and tissues. Anti-WNT1 neutralizing antibody showed its growth inhibitory effect on HCC cell lines but not on normal hepatocytes, with reduced β-catenin’s transcriptional activity (Fig. 4).

**Wnt antagonists**

SFRPs, WIFs, and DKKs are the secreted Wnt signaling antagonists. SFRP-1 and Wnt inhibitory factor 1 (WIF1) inhibit Wnt signaling by directly binding to Wnt ligands. The

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**Figure 4.** Manipulating Wnt signaling. Illustration of components and processes of Wnt signal transduction as druggable targets for liver cancer treatment. See the text for detail. Created with BioRender.com. GPC3, glypican-3; LRPs, lipoprotein receptor-related protein; FZD, frizzled; DKK1, Dickkopf 1; ROR, receptor tyrosine kinase-like orphan receptor; RYK, receptor tyrosine kinase; PORCN, porcupine; ER, endoplasmic reticulum; GSK-3β, glycogen synthase kinase 3β; CK1α, casein kinase 1α; APC, adenomatous polyposis coli; TCF, T-cell factor; CBP, CREB binding protein; LEF, lymphoid enhancer-binding factor; peg-IFN, pegylated-Interferon-α2a; RanBP3, Ran-binding protein 3.
fusion proteins WIF1-Fc and SFRP1-Fc were constructed by adding the Fc fragment of human immunoglobulin (Ig) G1 to WIF1 and SFRP1, respectively (Fig. 4). The fusion proteins exert potent anti-tumor activity by downregulating E2F transcription factor 1 (E2F1), cyclin D1, and c-Myc, increasing apoptosis of HCC cells and impairing tumor vascularization. DKK1 was initially considered a β-catenin-dependent tumor suppressor. Several studies have shown that DKK1 promotes tumor cell proliferation, which may be due to DKK1-induced endocytosis of LRP and subsequent activation of the Wnt/PCP signaling pathway. DKK-01 is a humanized monoclonal antibody targeting DKK1 in phase I/II clinical trial for HCC (Fig. 4). Phase I investigated the safety of DKK-01 as a single agent and in combination with sorafenib to treat HCC. Phase II explores the anti-tumor activity and safety of DKK-01 in patients with advanced HCC.

FZD receptors

The FZD receptors are promising therapeutic targets for HCC. The anti-FZD antibody can effectively reduce the HCC tumor growth by blocking the activation of FZD receptors on the Wnt signaling. FZD decoy receptor OMP-54F28 (iparfitccept) is a recombinant fusion protein that binds to a human IgG1 Fc fragment of FZD8, which acts synergistically with chemotherapeutic agents (Fig. 4). A phase I dose-escalation clinical trial evaluated the safety, tolerability, and pharmacokinetics of OMP-54F28 when combined with sorafenib. Secreted FZD7 (sFZD7) is the extracellular domain of FZD7, expressed and purified from Escherichia coli. sFZD7 binding to WNT3 decreased the transcriptional activity of β-catenin/TCF4 and inhibited the growth of HepG2, Hep40, and Huh7. In combination with doxorubicin, sFZD7 inhibited the expression of c-Myc/MYC, Cyclin D1/CCND1, and Survivin/BIRC5, reduced the phosphorylation levels of AKT and ERK1/2, inhibited the growth of Huh7 xenograft tumors, and acted as a chemosensitizer. OMP-54F28 is entering phase I clinical trials, while sFZD7 remains in preclinical studies (Fig. 4).

FZD antibody OMP-18R5 (vantictumab) is a monoclonal antibody directly binding to FZD receptors, which blocks the binding of Wnt ligands to FZD 1, 2, 3, 5, 7, and 8, which inhibits β-catenin-mediated transactivation (Fig. 4). In patient-derived xenograft models, OMP-18R5 combined with chemotherapeutic agents synergistically inhibited the development of several cancers. However, like PORCN inhibitors, OMP-18R5 has the same risk of impairing bone homeostasis. In a dose-escalation clinical trial of OMP-18R5, one patient developed bone degeneration, controllable with zoledronic acid. The skeletal toxicity appeared to be manageable and reversible.

LRP co-receptors

Salinomycin (SAL), isolated from Streptomyces albus, is a monocarboxylic polyether ionophore antibiotic. SAL blocks Wnt-induced LRP phosphorylation and leads to LRP protein degradation, destabilizing the Wnt/FZD/LRP complex and inhibiting the Wnt/β-catenin signaling (Fig. 4). SAL effectively inhibits β-catenin expression in HepG2/C3a cell line. SAL also inhibits the migration and invasiveness of liver cancer stem cells through the Wnt/β-catenin signaling suppression.

Tankyrase (TNKS)

TNKS mediates PARsylation and subsequent degradation of AXIN via the ubiquitin-proteasome pathway, which in turn disrupts the β-catenin destruction complex. Subsequently, the released β-catenin enters the nucleus to transactivate Wnt target genes. TNKS is overexpressed in many cancers, including HCC, gastric cancer, and colorectal cancer. The TNKS inhibitors XAV939, WXl-8, and NVP-TNKS656, attenuated Wnt/β-catenin signaling and inhibited the growth of HCC cells (Fig. 4). Moreover, TNKS inhibitors also suppressed HCC metastasis and invasion. However, there are no relevant clinical trials for TNKS inhibitors in HCC.

Nuclear export of β-catenin

As shown in Table 1 and Supplementary Table 1, pegylated-Interferon-α2a (peg-IFN), the first-line therapy for the HCV-infected, attenuates the recurrence of HCC (Fig. 4). Mechanistically, peg-IFN upregulates the expression of Ran-binding protein 3 (RanBP3), which enhances the nuclear export of β-catenin. Thus, it is likely that peg-IFN-induced β-catenin nuclear export is a mechanism delaying HCC and improving survival in HCV patients.
**β-catenin-mediated gene transactivation**

The small molecule ICG-001 inhibits the interaction between β-catenin and CREB binding protein (CREBBP/CBP) for suppression of β-catenin-mediated gene transactivation (Fig. 4). A phase Ib/IIa clinical trial of the ICG-001 derivative, PRI-724, targeting HCC has been terminated. Similar to ICG-001, PMED-1 disrupts β-catenin-CREBBP interaction and suppresses β-catenin target gene activation. PMED-1 inhibits HCC cell proliferation but not normal human hepatocytes. PKF118-310, PKF115-584, and CGP049090 are small-molecule inhibitors targeting the β-catenin-TCF complex (Fig. 4). These antagonists displayed the dose-dependent cytotoxicity in HepG2, Hep40, and Huh7 cell lines, with reduced cytotoxicity (10%) to normal hepatocytes. PKF118-310, PKF115-584, and CGP049090 downregulated β-catenin target genes (MYC, CCND1, and Survivin/BIRC5) and inhibited the growth of HepG2 xenografts. Similar to the mechanism of PKF118-

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<tr>
<td>CGK062</td>
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<tr>
<td>PMED-1</td>
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<td>PRI-724</td>
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<tr>
<td>Hydroxychloroquine</td>
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<td>Chloroquine</td>
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<td>Bafilomycin</td>
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<td>Concanamycin</td>
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<tr>
<td>CAR-GPC3 T cell</td>
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<tr>
<td>Anti-GPC3 antibody</td>
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<td>CIK with anti-GPC3</td>
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</tbody>
</table>

DKK1, Dickkopf 1; FZD, frizzed; NA, not available; PORCN, porcupine; TCF, T-cell factor; peg-IFN, pegylated-Interferon-α2a; CBP, CREB binding protein; v-ATPase, vacuolar-type ATPase.
310, PKF115-584, and CGP049090, FH535 inhibits β-catenin-mediated gene transactivation by interrupting the recruitment of nuclear receptor coactivator 2 (NCOA2)/GRIPI to the β-catenin transcriptional complex.166 It was shown that FH535 inhibits HCC cell proliferation by reducing cancer cell stemness.165

**β-catenin phosphorylation**

CGK062 promotes PKCα-mediated phosphorylation of β-catenin at Ser33/Ser37, which degrades β-catenin by the proteasome (Fig. 4).167 Consistently, CGK062 inhibited the expression of β-catenin target genes (CCND1, MYC, and AXIN2) and suppressed the growth of Wnt/β-catenin-activated HCC cells.167

**Cancer-specific targeting of Wnt signaling**

Given the pivotal role of Wnt signaling in the homeostasis and regeneration of multiple organs,168-170 broad-spectrum Wnt signaling inhibitors cause detrimental effects on the normal cells and organs. Therefore, cancer-specific Wnt signaling regulators may be attractive for Wnt signaling blockade therapy. TMEM9, an amplifier of Wnt/β-catenin signaling, promotes lysosomal protein degradation via v-ATPase, resulting in APC downregulation.46 TMEM9 is highly expressed in liver regeneration and HCC. Genetic ablation of TMEM9 inhibits HCC tumorigenesis with downregulation of Wnt/β-catenin signaling.46 Similarly, v-ATPase inhibitors, bafilomycin and concanamycin,171,172 also inhibit Wnt/β-catenin signaling without toxicity to normal cells and animals (Fig. 4, Table 1).45,46 Thus, molecular targeting of the TMEM9-v-ATPase axis can be used as cancer-specific Wnt/β-catenin blockade.

Glypican-3 (GPC3) is a proteoglycan binding to the FZD receptor and stimulates Wnt ligands-FZD interaction, resulting in the Wnt signaling activation (Fig. 4).173 GPC3 is specifically expressed in HCC but not in normal human liver tissue.174 The ectopic expression of GPC3 promotes the proliferation of HCC cells.175 HS20 (an anti-GPC3 monoclonal antibody) suppresses Wnt/β-catenin signaling via inhibiting the interaction of Wnt3a with the GPC3.176 In xenograft mouse models, HS20 inhibited HCC progression without apparent concomitant toxicity.176 To date, including CAR-GPC3 T cells or anti-GPC3 antibodies, 33 clinical trials related to GPC3 for HCC treatment were registered (https://clinicaltrials.gov/) (Table 1, Supplementary Table 1).

**Concluding remarks**

Wnt signaling activation plays a pivotal role in liver regeneration, metabolic zonation, liver diseases, and liver cancer. Aberrantly hyperactivated Wnt signaling promotes liver tumorigenesis and progression, often in conjunction with liver diseases. Although direct targeting of Wnt signaling sounds attractive as cancer therapy, given the crucial roles of Wnt signaling in tissue homeostasis and regeneration, severe adverse effects from Wnt blockade are inevitable. Nonetheless, an in-depth understanding of the biology of Wnt signaling in liver cancer and exploring cancer-specific Wnt signaling regulators are expected to identify molecular targets specific to liver cancer, which may overcome the current limitations of Wnt signaling inhibitors, and further improve therapeutic strategies of liver cancer treatment.

**Authors’ contribution**

G.Z. and J.I.P. wrote the manuscript.

**Acknowledgements**

We apologize for not including all relevant studies in the field due to limited space. This work was supported by the Cancer Prevention and Research Institute of Texas (RP200315 to J.I.P.) and the National Cancer Institute (CA193297 and CA256207 to J.I.P.).

**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).

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Development and prognosis of hepatocellular carcinoma in patients with diabetes

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The incidence of diabetes mellitus and hepatocellular carcinoma (HCC) has been increasing worldwide during the last few decades, in the context of an increasing prevalence of obesity and non-alcoholic fatty liver disease (NAFLD). Epidemiologic studies have revealed that patients with diabetes have a 2- to 3-fold increased risk of developing HCC, independent of the severity and cause of the underlying liver disease. A bidirectional relationship exists between diabetes and liver disease: advanced liver disease promotes the onset of diabetes, and HCC is an important cause of death in patients with diabetes; conversely, diabetes is a risk factor for liver fibrosis progression and HCC development, and may worsen the long-term prognosis of patients with HCC. The existence of close interconnections among diabetes, obesity, and NAFLD causes insulin resistance-related hyperinsulinemia, increased oxidative stress, and chronic inflammation, which are assumed to be the underlying causes of hepatocarcinogenesis in patients with diabetes. No appropriate surveillance methods for HCC development in patients with diabetes have been established, and liver diseases, including HCC, are often overlooked as complications of diabetes. Although some antidiabetic drugs are expected to prevent HCC development, further research on the optimal use of antidiabetic drugs aimed at hepatoprotection is warranted. Given the increasing medical and socioeconomic impact of diabetes on HCC development, diabetologists and hepatologists need to work together to develop strategies to address this emerging health issue. This article reviews the current knowledge on the impact of diabetes on the development and progression of HCC. (Clin Mol Hepatol 2023;29:51-64)

Keywords: Hepatocellular carcinoma; Diabetes mellitus; Insulin resistance; Hyperinsulinemia; Non-alcoholic fatty liver disease

INTRODUCTION

The incidence and mortality of liver cancer have been continuously increasing during the last decades. Hepatocellular carcinoma (HCC), the most common form of primary liver cancer, develops in the context of chronic liver disease in 70–90% of the cases. The main causes of the underlying liver diseases are persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) and alcohol abuse. However, in recent years, non-alcoholic fatty liver disease (NAFLD) and its more active form, non-alcoholic steatohepatitis (NASH), have emerged as new risk factors for HCC and are replacing viral- and alcohol-related liver diseases as major pathogenic promoters, particularly in developed countries. NAFLD is a hepatic manifestation of metabolic syndrome, which is strongly associated with overweight or obesity, hypertension, dyslipidemia, and diabetes mellitus.

Type 2 diabetes mellitus (T2DM) is a disease characterized...
by hyperglycemia, hyperinsulinemia, and insulin resistance, with a tremendous impact on human health worldwide. Epidemiologic evidence suggests that patients with diabetes have an increased risk of many kinds of cancer, including breast, pancreatic, lung, colorectal, and kidney cancers. In particular, an important relationship between the presence of diabetes and a higher incidence of HCC has been confirmed. This relationship occurs in association with obesity, impaired insulin sensitivity, and NAFLD, which are well-established risk factors for HCC development. Independent of the presence of cirrhosis or the cause of the underlying liver disease, patients with diabetes have a 2- to 3-fold higher risk of developing HCC than individuals without diabetes. A longer duration of diabetes may also be associated with an incremental increase in the risk of HCC and diabetes is an independent risk factor associated with reduced overall survival and disease-free survival in patients with HCC. In addition, the proportion of diabetes among patients with HCC of nonviral etiology has continued to increase considerably during the last two decades. Given the rapid increase in the global incidence of HCC and diabetes, hepatologists and diabetologists must recognize the strong link between the two diseases and appropriately manage diabetes to prevent the development of liver diseases and reduce the risk of HCC.

This review article summarizes the current knowledge on the impact of diabetes on the development and progression of HCC from an epidemiologic and pathophysiologic perspective. In addition, the relationship between diabetes medications and the risk of HCC development is also discussed.

IMPACT OF DIABETES ON LIVER DISEASE PROGRESSION

There is a known close relationship between chronic liver disease and diabetes. In a recent meta-analysis involving 58 studies with 9,705 patients with cirrhosis, the overall prevalence of diabetes was 31%, with the highest prevalence in patients with NAFLD (56%), followed by patients with cryptogenic liver disease (51%), HCV infection (32%), and alcoholic liver disease (27%). Given that the liver plays a pivotal role in energy homeostasis and glucose metabolism, the close link between liver disease and diabetes is convincing.

The most common chronic liver disease observed in patients with diabetes is NAFLD. As a metabolic syndrome component, T2DM can promote NAFLD. According to a recent meta-analysis involving 80 studies from 20 countries, conducted by Younossi et al., the global prevalence of NAFLD and NASH among patients with T2DM was 55.5% (95% confidence interval [CI], 47.3–63.7%) and 37.3% (95% CI, 24.7–50.0%), respectively. Given that the overall global prevalence of NAFLD was reported to be approximately 25%, diabetes is clearly associated with the incidence and progression of NAFLD. The presence of insulin resistance and diabetes is considered a risk factor for more severe liver disease in NAFLD, even in patients with normal serum levels of alanine aminotransferase (ALT). Meanwhile, NAFLD itself is associated with a 2- to 5-fold increased risk of diabetes development after correcting for various lifestyle and metabolic confounders.

Diabetes is a risk factor for the development and progression of liver fibrosis, and a strong relationship exists between insulin resistance and liver fibrosis progression. Several large cohort studies have shown that diabetes is associated with a 2- to 2.5-fold increased risk of cirrhosis, mainly due to NAFLD, independent of other metabolic syndrome components. In contrast, glucose metabolism is altered in patients with advanced cirrhosis. Once cirrhosis is established, hyperglycemia may develop in up to 20% of patients within 5 years. Furthermore, up to 80% of patients with cirrhosis may have insulin resistance, and between 20% and 60% will develop diabetes. In patients with cirrhosis, hepatic insulin uptake and clearance are reduced owing to decreased liver cell mass and portosystemic venous collaterals, leading to impaired glucose tolerance and hyperinsulinemia. In summary, diabetes and chronic liver disease, particularly NAFLD, can affect each other synergistically, causing the other condigths
EPIDEMIOLOGIC STUDIES ON THE DEVELOPMENT OF HCC IN PATIENTS WITH DIABETES

Diabetes and cancer risk

The global prevalence of T2DM in 2019 was estimated to be approximately 9.3%, and the incidence is expected to continue to increase. In parallel, the total number of deaths attributable to cancer is estimated to increase over time. As there is strong evidence of a gradual increase in cancer risk and mortality with increasing incidence of diabetes, diabetes is considered a risk factor for the development of various cancers. In an umbrella review of the evidence across meta-analyses of observational studies on the association of diabetes with the risk of cancer development, the relative risk (RR) of HCC was 2.31 (95% CI, 1.87–2.84), the highest of the 20 cancer types.

Prediabetes, including impaired fasting glucose and impaired glucose tolerance, is also associated with an increased risk of cancer. A meta-analysis of 16 prospective cohort studies with 891,426 participants revealed that prediabetes was associated with an increased overall cancer risk (RR, 1.15; 95% CI, 1.06–1.23), with a particularly high risk of HCC (RR, 2.01; 95% CI, 1.45–2.79). These findings suggest that diabetes, and even prediabetic hyperinsulinemia or hyperglycemia, may be strongly associated with the development of HCC.

Diabetes and HCC risk

The link between diabetes and HCC was first reported approximately 40 years ago. Lawson et al. observed a 4-fold excess of patients with diabetes among patients with HCC, in a case-control study involving 105 patients with HCC and equal numbers of age- and sex-matched controls. Subsequently, to date, various case-control, prospective cohort, and meta-analysis studies have shown a positive association between diabetes and an increased risk of HCC.

The principal observational and meta-analysis studies examining the association between diabetes and the risk of HCC are listed in Table 1. For example, El-Serag et al. demonstrated that diabetes was significantly associated with the risk of incident HCC (hazard ratio [HR], 2.5; 95% CI, 1.9–3.2) in a meta-analysis of 13 cohort studies. The results were relatively consistent in different populations, different geographic locations, and a variety of control groups, and the association between HCC and diabetes was independent of alcohol use or viral hepatitis. In a meta-analysis of 25 cohort studies, Wang et al. showed that diabetes was associated with an increased incidence of HCC (summary RR, 2.01; 95% CI, 1.61–2.51) compared with the absence of diabetes, and the association was independent of geographic location, alcohol consumption, history of cirrhosis, and HBV or HCV infection.

In another meta-analysis involving 17 case-control studies and 32 cohort studies, Wang et al. confirmed that the combined risk estimate of all studies showed a statistically significant increased risk of HCC among individuals with diabetes (RR, 2.31; 95% CI, 1.87–2.84), independent of several confounding factors and metabolic variables. Furthermore, Chen et al. conducted a meta-analysis of 21 cohort studies and identified a total of 2,528 HCC cases in 35,202 participants. The summary RR of HCC with diabetes was 1.86 (95% CI, 1.49–2.31) in patients with chronic liver disease and 1.93 (95% CI, 1.35–2.76) in patients with cirrhosis, and subgroup analyses indicated that the positive associations were independent of geographic location, follow-up duration, and confounding factors such as smoking, alcohol use, and body mass index. In a recent large population-based cohort study including 50,284 men and 120,826 women enrolled in 1986 and followed up through 2012, Simon et al. documented that diabetes was associated with an increased risk of HCC (HR, 4.59; 95% CI, 2.98–7.07), as was an increasing diabetes duration. Compared with individuals without diabetes, the multivariable HR for HCC was 2.96 (95% CI, 1.57–5.60) in those with a diabetes duration of <2 years, 6.08 (95% CI, 2.96–12.50) in those with a diabetes duration of <10 years, and 7.52 (95% CI, 3.88–14.58) in those with a diabetes duration of ≥10 years. These vast epidemiologic findings strongly suggest that diabetes has a considerable impact on the risk of HCC development, independent of various confounding factors.

Diabetes has a significant impact on hepatocarcinogenesis, particularly in patients with NAFLD. In a recent retrospective cohort study in patients with NAFLD diagnosed at 130 facilities of the Veterans Administration, Kanwal et al. reported that 253 of the 271,906 patients developed HCC during a mean follow-up period of 9 years, and diabetes conferred the highest risk of progression to HCC among the metabolic fac-
### Table 1. Principal observational studies and meta-analysis on the association between type 2 diabetes mellitus and risk of hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Description of the study</th>
<th>Subjects</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davila et al. (2005)</td>
<td>USA</td>
<td>Population-based case-control study</td>
<td>2,061 HCC cases and 6,183 controls</td>
<td>AOR, 2.87 (95% CI, 2.49–3.30)</td>
</tr>
<tr>
<td>Adami et al. (1996)</td>
<td>Sweden</td>
<td>Hospital-based cohort study</td>
<td>153,852 with DM</td>
<td>SIR, 4.1 (95% CI, 3.8–4.5)</td>
</tr>
<tr>
<td>Wideroff et al. (1997)</td>
<td>Denmark</td>
<td>Population-based cohort study</td>
<td>109,581 with DM</td>
<td>SIR, 4.0 (95% CI, 3.5–4.6)</td>
</tr>
<tr>
<td>Coughlin et al. (2004)</td>
<td>USA</td>
<td>Population-based cohort study</td>
<td>467,922 men and 588,321 women without history of cancer</td>
<td>RR, 2.19 (95% CI, 1.76–2.72) for male</td>
</tr>
<tr>
<td>Johnson et al. (2011)</td>
<td>Canada</td>
<td>Population-based cohort study</td>
<td>185,100 with DM and 185,100 without DM</td>
<td>HR, 2.53 (95% CI, 1.93–3.31)</td>
</tr>
<tr>
<td>Lai et al. (2012)</td>
<td>Taiwan</td>
<td>Population-based cohort study</td>
<td>19,349 with DM and 77,396 without DM</td>
<td>HR, 1.73 (95% CI, 1.47–2.03)</td>
</tr>
<tr>
<td>Schlesinger et al. (2013)</td>
<td>European countries</td>
<td>Population-based cohort study</td>
<td>363,426 participants without cancer</td>
<td>RR, 2.17 (95% CI, 1.36–3.47)</td>
</tr>
<tr>
<td>Koh et al. (2013)</td>
<td>Singapore</td>
<td>Population-based cohort study</td>
<td>63,257 middle-aged and older individuals</td>
<td>HR, 2.14 (95% CI, 1.69–2.71)</td>
</tr>
<tr>
<td>Setiawan et al. (2014)</td>
<td>USA</td>
<td>Population-based cohort study</td>
<td>168,679 multiethnic individuals</td>
<td>RR, 2.62 (95% CI, 2.13–3.23)</td>
</tr>
<tr>
<td>Simon et al. (2018)</td>
<td>USA</td>
<td>Population-based cohort study</td>
<td>50,284 men and 120,826 women</td>
<td>HR, 4.59 (95% CI, 2.98–7.07)</td>
</tr>
<tr>
<td>El-Serag et al. (2004)</td>
<td>USA</td>
<td>Prospective cohort study</td>
<td>173,643 with DM and 650,620 without DM</td>
<td>HR, 2.16 (95% CI, 1.86–2.52)</td>
</tr>
<tr>
<td>Inoue et al. (2006)</td>
<td>Japan</td>
<td>Prospective cohort study</td>
<td>97,771 individuals</td>
<td>HR, 2.24 (95% CI, 1.64–3.04) for male; HR, 1.94 (95% CI, 1.00–3.73) for female</td>
</tr>
<tr>
<td>Lai et al. (2006)</td>
<td>Taiwan</td>
<td>Prospective cohort study</td>
<td>5,732 with DM and 49,184 without DM</td>
<td>HR, 1.84 (95% CI, 1.10–3.07)</td>
</tr>
<tr>
<td>Atchison et al. (2011)</td>
<td>USA</td>
<td>Prospective cohort study</td>
<td>594,815 men with DM and 3,906,763 men without DM</td>
<td>RR, 1.95 (95% CI, 1.82–2.09)</td>
</tr>
<tr>
<td>El-Serag et al. (2006)</td>
<td>-</td>
<td>Meta-analysis</td>
<td>13 case-control studies and 13 cohort studies</td>
<td>HR, 2.5 (95% CI, 1.9–3.2)</td>
</tr>
<tr>
<td>Yang et al. (2011)</td>
<td>-</td>
<td>Meta-analysis</td>
<td>28 prospective studies</td>
<td>RR, 1.87 (95% CI, 1.15–2.27)</td>
</tr>
<tr>
<td>Wang et al. (2012)</td>
<td>-</td>
<td>Meta-analysis</td>
<td>25 cohort studies</td>
<td>SRRs, 2.01 (95% CI, 1.61–2.51)</td>
</tr>
<tr>
<td>Wang et al. (2012)</td>
<td>-</td>
<td>Meta-analysis</td>
<td>49 studies (32 cohorts and 17 case-controls)</td>
<td>RR, 2.31 (95% CI, 1.87–2.84)</td>
</tr>
<tr>
<td>Chen et al. (2015)</td>
<td>-</td>
<td>Meta-analysis</td>
<td>21 cohort studies</td>
<td>SRRs, 1.86 (95% CI, 1.49–2.31)</td>
</tr>
<tr>
<td>Kanwal et al. (2020)</td>
<td>USA</td>
<td>Retrospective cohort study (NAFLD)</td>
<td>271,906 with NAFLD</td>
<td>HR, 2.77 (95% CI, 2.03–3.77)</td>
</tr>
<tr>
<td>Tan et al. (2019)</td>
<td>-</td>
<td>Meta-analysis (HBV)</td>
<td>7 studies (5 cohorts and 2 case-controls)</td>
<td>HR, 1.77 (95% CI, 1.28–2.47)</td>
</tr>
<tr>
<td>Dyal et al. (2016)</td>
<td>-</td>
<td>Meta-analysis (HCV)</td>
<td>9 studies (7 cohorts and 2 case-controls)</td>
<td>HR, 1.73 (95% CI, 1.30–2.30)</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; AOR, adjusted odds ratio; CI, confidence interval; DM, diabetes mellitus; SIR, standardized incidence ratios; RR, relative risk; HR, hazard ratio; SRRs, summary relative risks; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus.
tors (HR, 2.77; 95% CI, 2.03–3.77). On the other hand, diabetes also plays an important role in HCC associated with viral hepatitis. In a meta-analysis of five cohort studies and two case-control studies in patients with HBV, Tan et al.\textsuperscript{54} demonstrated that the diabetes cohort had a higher incidence of HCC (pooled HR, 1.77; 95% CI, 1.28–2.47) than individuals without diabetes. In a meta-analysis of nine studies (seven cohort studies and two case-control studies) in patients with HCV, Dyal et al.\textsuperscript{55} found that diabetes was closely associated with an increased risk of HCC (HR, 1.73; 95% CI, 1.30–2.30), independent of age, sex, obesity, hypertension, smoking, alcohol intake, serum liver enzyme levels, albumin, lipids, platelet count, and presence of cirrhosis or hepatic steatosis. Furthermore, Arase et al.\textsuperscript{56} documented that diabetes caused a 1.73-fold increase in the HCC risk even after the termination of interferon therapy, in a retrospective cohort study involving 4,302 patients with HCV treated with interferon. In addition, in a recent meta-analysis involving 30 cohort studies, Váncsa et al.\textsuperscript{15} demonstrated that diabetes was a significant risk factor for HCC in patients with HCV treated with direct-acting antivirals (adjusted HR, 1.31; 95% CI, 1.06–1.62). These findings indicate that diabetes is an independent risk factor for viral hepatitis-related HCC, even after viral elimination.

**PATHOPHYSIOLOGIC MECHANISMS LINKING DIABETES WITH HCC RISK**

Although the detailed mechanisms of carcinogenesis in patients with diabetes remain unclear, insulin resistance-related hyperinsulinemia and DNA damage due to increased oxidative stress are assumed to be the main causes.\textsuperscript{57} Persistent hyperinsulinemia increases the production of insulin-like growth factor (IGF)-binding proteins, which, in turn, increase the bioavailability of IGF-1 produced by the liver. Elevated blood insulin and IGF-1 levels activate phosphoinositide-3-kinase (PI3K)/AKT/mammalian target of rapamycin signaling, a key pathway involved in fatty liver-related carcinogenesis,\textsuperscript{58,59} which promotes hepatic cell proliferation and inhibits apoptosis.\textsuperscript{60} Furthermore, hyperglycemia increases oxidative stress production through excess glucose oxidation in mitochondria. Many patients with diabetes have metabolic factors (e.g., obesity or dyslipidemia) and develop fatty liver, in which fatty acid oxidation facilitates the generation of reactive oxygen species and increases oxidative stress production. Oxidative stress is a known cause of vascular damage in diabetes and also induces genetic mutations through oxidative DNA damage, leading to carcinogenesis.\textsuperscript{57} Furthermore, liver fat accumulation induces chronic inflammation and increases the production of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), and nuclear factor-kB, which may be involved in hepatocarcinogenesis.\textsuperscript{51} In addition, alterations in the gut microbiota in patients with obesity and diabetes have also been implicated in NASH development and hepatocarcinogenesis.\textsuperscript{62} Thus, a complex combination of direct and indirect mechanisms is postulated to promote hepatocarcinogenesis in patients with diabetes. The putative pathophysiologic mechanisms that may link diabetes and HCC are schematically summarized in Figure 1.

Obesity, diabetes, and metabolic syndrome may accelerate the progression of liver disease in patients with viral hepatitis as well as NAFLD. Diabetes and hepatitis virus infection synergistically induce the development of HCC. HCV infection itself is known to be associated with insulin resistance, which contributes to the progression of underlying liver fibrosis and the development of HCC by accelerating necroinflammation and oxidative stress in the liver.\textsuperscript{27} Mechanistically, the core protein of the virus promotes insulin resistance by inducing the degradation of insulin receptor substrate-1.\textsuperscript{63} In addition, HCV proteins directly associate with mitochondria and endoplasmic reticulum, promoting oxidative stress.\textsuperscript{64} On the other hand, the link between HBV and metabolic syndrome or insulin resistance remains inconclusive; HBV infection itself appears to protect against steatosis, metabolic syndrome, and insulin resistance.\textsuperscript{65}

**INFLUENCE OF DIABETES ON HCC PROGNOSIS**

**Impact of HCC as a cause of death in patients with diabetes**

Diabetes is an important risk factor for HCC development, whereas HCC is an important cause of death in patients with diabetes. In an analysis of individual-participant data on 123,205 deaths among 820,900 people in 97 prospective studies, the risk of HCC mortality was 2.16 times higher in patients with diabetes (95% CI, 1.62–2.88) than in those without diabetes, and the mortality risk from HCC of patients with diabetes was higher than that from all other cancers.\textsuperscript{66} In addi-
tion, Nakamura et al. investigated the principal causes of death among 45,708 patients with diabetes who died in 241 hospitals throughout Japan during 2001–2010 and found that the most frequent cause of death was malignant neoplasms (38.3%), with liver cancer (6.0%) being the second leading cause of cancer death after lung cancer (7.0%). Therefore, HCC should be recognized as both an important cause of death and an important comorbidity that cannot be overlooked in patients with diabetes.

**Impact of diabetes on the prognosis of patients with HCC**

Various studies have been published on the impact of diabetes on the prognosis of patients with HCC, most of which state that diabetes itself worsens the prognosis of HCC. In a nationwide prospective study including 512,869 adults from 10 regions in China, Bragg et al. documented that preexisting diabetes was significantly associated with HCC-specific mortality (RR, 1.88; 95% CI, 1.39–2.55) and even all-cause death (RR, 1.38; 95% CI, 1.13–1.48), compared with the absence of diabetes. In another meta-analysis, diabetes was positively associated with HCC mortality (summary RR, 1.56; 95% CI, 1.30–1.87). In addition, a meta-analysis of seven cohort studies found a statistically significant increased risk of HCC mortality (RR, 2.43; 95% CI, 1.66–3.55) in individuals with diabetes. In another meta-analysis involving 20 studies with a total of 9,727 patients with HCC, Wang et al. demonstrated that diabetes was associated with poor overall survival (adjusted HR, 1.55; 95% CI, 1.27–1.91). They also revealed that diabetes was associated with poor overall survival even in patients with HCC who have undergone curative therapy, including hepatic resection or nonsurgical treatment such as radiofrequency ablation. In addition, Liu et al. demonstrated that diabetes was an independent risk factor for time to progression (HR, 1.29; 95% CI, 1.04–1.60) and cancer-specific mortality (HR, 1.24;
HCC SURVEILLANCE IN PATIENTS WITH DIABETES

The current regional guidelines recommend HCC surveillance only in patients with cirrhosis.\(^7\)\(^{-7}\)\(^5\) However, up to 50% of cases of NAFLD-driven HCC, which is closely associated with metabolic syndrome including diabetes, occur in patients without cirrhosis, possibly owing to its unique nature of arising from lipotoxicity-mediated chronic inflammation.\(^4\) Some reports suggest that an annual incidence of 1.5–2.0% would guarantee the cost-effectiveness of HCC surveillance. As mentioned above, diabetes may increase the risk of HCC by approximately 2- to 3-fold; however, this is much lower than the 24-fold increased risk caused by HBV or HCV.\(^7\)\(^9\) In addition, the annual incidence of HCC in patients with diabetes is estimated to be <0.1%,\(^19\) which is far below the threshold for efficient surveillance. Therefore, establishing strategies for efficient HCC surveillance in patients with diabetes has been challenging. As nonviral HCC tends to be diagnosed at an advanced stage\(^25\) and diabetes is a factor associated with HCC detection over the Milan criteria,\(^77\) there is an urgent need for a method for detecting HCC while the disease is in a treatable state in patients with diabetes.

To date, several HCC risk prediction models have been reported. Si et al.\(^7\) established the Korean DM-HCC risk score using data from 3,544 patients with diabetes without viral hepatitis or alcoholic liver disease. In their study, three parameters (age >65 years, low triglyceride levels, and high gamma-glutamyl transferase levels) were independently associated with an increased risk of HCC, and the weighted sum of the scores from these three parameters predicted the 10-year incidence of HCC with an area under the receiver operating characteristic curve (AUROC) of 0.86. Li et al.\(^7\) developed an HCC risk scoring system considering age, sex, smoking, hemoglobin A1c level, ALT level, presence of cirrhosis or viral hepatitis, antidiabetic or antihyperlipidemic medications, and total/high-density lipoprotein cholesterol ratio, using the Taiwan National Diabetes Care Management Program database including 31,723 Chinese patients with T2DM. The AUROC for the 3-, 5-, and 10-year HCC risk was 0.81, 0.80, and 0.77, respectively. These two models were based on the Cox proportional hazard model. Meanwhile, Rau et al.\(^80\) developed an artificial neural network model for predicting HCC occurrence within 6 years of diabetes diagnosis by considering age, sex, hyperlipidemia, and chronic liver diseases, with an AUROC of 0.873. They analyzed 515 patients with diabetes who developed HCC after the diabetes diagnosis and compared them with matched 1,545 controls from the National Health Insurance Research Database of Taiwan.

Because liver fibrosis is the most important predictor of HCC development in patients with chronic liver disease, non-invasive fibrosis markers are a potential tool for risk stratification of HCC. Grecian et al.\(^85\) tested the ability of individual fibrosis scores, including the enhanced liver fibrosis test, aspartate aminotransferase (AST)-to-platelet ratio index, AST-to-ALT ratio, NAFLD fibrosis score, and fibrosis-4 (FIB-4) index, to predict 11-year incident cirrhosis/HCC in a community cohort of 1,066 people with T2DM aged 60–75 years. All scores were significantly associated with incident liver-related events; however, they showed poor ability as a risk stratification tool, with low positive predictive values (5–46%) and
high false-negative and false-positive rates (up to 60% and 77%, respectively). Recently, we investigated the best criteria for identifying candidates for HCC surveillance among patients with diabetes. The study included 239 patients with T2DM and nonviral HCC with >5 years of follow-up at diabetes clinics in 81 hospitals in Japan before the HCC diagnosis and 3,277 patients with T2DM without HCC from a prospective cohort study as controls. Multivariate logistic regression analyses showed that the FIB-4 index was an outstanding predictor of HCC development, with an AUROC of 0.811 for predicting the 5-year HCC incidence. Furthermore, an FIB-4 cutoff value of 3.61 helped identify high-risk patients, with a corresponding annual HCC incidence rate of 1.1%. These findings suggest the importance of setting appropriate cutoff values to identify high-risk cases and the utility of the simple calculation of the FIB-4 index as the first step toward HCC surveillance in patients with diabetes. A prospective study is warranted to validate the efficacy of an FIB-4-based surveillance strategy.

MEDICATIONS FOR DIABETES MELLITUS AND HCC RISK

As previously described, diabetes has been epidemiologically proven to increase the risk of HCC development; however, whether appropriate glycemic control can prevent HCC development is controversial. Recently, Luo et al. found that a higher dietary diabetes risk reduction score, reflecting better adherence to a dietary therapy for T2DM prevention, was independently associated with a significantly lower risk of HCC among 137,608 USA participants after adjusting for major known risk factors for HCC, indicating that diabetes prevention may lead to a reduced risk of HCC development. Similarly, increasing evidence has suggested the potential hepatoprotective effects of some antidiabetic drugs.

Metformin is a biguanide compound that improves insulin resistance by targeting the enzyme adenosine monophosphate-activated protein kinase, which induces the muscle uptake of glucose from the blood. In 2005, it was first reported that the use of metformin in patients with T2DM may reduce their risk of cancer. Since then, numerous studies have been conducted on the preventive effect of metformin on the development of various cancers, including HCC. Singh et al. conducted a meta-analysis involving 10 studies with 22,650 cases of HCC in 334,307 patients with T2DM and showed an overall 50% reduced risk of incident HCC among metformin-treated patients (adjusted odds ratio [OR], 0.50; 95% CI, 0.34–0.73). Importantly, the protective effect of metformin remained significant after adjusting for the effect of other antidiabetic medications. Furthermore, a recent meta-analysis by Li et al. showed that metformin use was significantly associated with a decreased risk of HCC in patients with T2DM (OR, 0.59; 95% CI, 0.51–0.68) and even with a decreased all-cause mortality in patients with diabetes and HCC (HR, 0.74; 95% CI, 0.66–0.83). In another meta-analysis, Zhou et al. also documented that metformin significantly prolonged the survival of patients with HCC and T2DM even after the curative treatment of HCC. Considering these results, metformin use may reduce the risk of HCC development by approximately 50%. However, in these studies, metformin was not administered for the prevention of HCC but for the treatment of diabetes, leading to various biases. Home et al. and Ma et al. reported that two randomized controlled trials in their meta-analysis did not show a protective effect of metformin against HCC development. Singh et al. also conducted a post hoc analysis of randomized controlled trials, and their results did not reveal any significant association between antidiabetic medication use and the risk of HCC. Thus, further validation based on evidence from randomized clinical trials is warranted.

Insulin is a potent mitogen associated with the upregulation of various growth factors that stimulate several signaling pathways related to cell proliferation and apoptosis inhibition. Epidemiologic evidence indicates that the insulin secretion rate influences cancer risk or prognosis, and patients with diabetes treated with insulin have a higher risk of HCC. Singh et al. found in their meta-analysis that insulin use was associated with an increased risk of HCC (OR, 2.61; 95% CI, 1.46–4.65). Schlesinger et al. conducted a prospective analysis involving 363,426 patients with diabetes and demonstrated that treatment with insulin conferred the highest risk of HCC (RR, 5.25, 95% CI, 2.93–9.44), whereas no association was observed in participants without insulin treatment. Likewise, in a large population-based study from Italy conducted by Bosetti et al., an increased risk of HCC was found with insulin use (OR, 3.73; 95% CI, 2.52–5.51), with a higher risk associated with a longer treatment duration. Although further validation is needed to clarify the true relationship between insulin use and hepatocarcinogenesis, epidemiologic and bi-
ological evidence suggests that insulin may have some hepatocarcinogenic effect.

The evidence for other classes of antidiabetic medications is limited and often inconsistent. Thiazolidinediones induce insulin sensitization and enhance glucose metabolism by activating peroxisome proliferator-activated receptor gamma. In a randomized controlled trial, pioglitazone, a thiazolidinedione, has been shown to improve the pathogenesis of NASH, a common complication of diabetes. However, according to several recent meta-analyses, the potential impact of thiazolidinediones on the development of HCC remains controversial. Similar to insulin, sulfonylureas (oral insulin secretagogues) are associated with an increased risk of cancer, including HCC. Although sulfonylureas are known to potentially increase the risk of HCC, different drug generations have shown inconsistent results.

In a large cohort study including 108,920 Taiwanese patients with newly diagnosed T2DM, Chang et al. observed a significantly increased risk of HCC in users of first- and second-generation sulfonylureas (adjusted OR, 1.40; 95% CI, 1.19–1.66), but no increased risk of HCC in users of the third-generation drug glimepiride. Dipeptidyl peptidase-4 (DPP-4) inhibitors work by increasing the circulating levels of incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide, leading to the enhancement of insulin secretion and inhibition of glucagon secretion. Although experimental data indicate that DPP-4 inhibitors may reduce the risk of HCC development, clinical evidence on the relationship between DPP-4 inhibitors and HCC development remains scarce. GLP-1 receptor agonists such as liraglutide and semaglutide have been reported to reduce body weight and improve hepatic histology in NASH. However, no clinical evidence exists on whether GLP-1 receptor agonists can prevent the occurrence of HCC. A new class of oral hypoglycemic agents, sodium-glucose cotransporter 2 (SGLT2) inhibitors, can attenuate glucose reabsorption in the proximal tubule, leading to plasma glucose reduction. SGLT2 inhibitors have been reported to reduce hepatic fat content in patients with NAFLD; however, there are no clinical data on whether they have a protective effect against hepatocarcinogenesis in patients with diabetes.

CONCLUSIONS

Diabetes mellitus, a disease characterized by hyperglycemia, hyperinsulinemia, and insulin resistance, has attracted large attention for its systemic complications. Although liver diseases, including NAFLD, NASH, cirrhosis, and HCC, are important complications of diabetes, they are often overlooked. To date, extensive epidemiologic and preclinical evidence has supported a robust association between diabetes and liver diseases, including HCC. In addition, a bidirectional relationship exists in that advanced liver disease may induce the onset of diabetes and diabetes is a recognized risk factor for the development and progression of liver disease and HCC. The association between these two diseases is complex, and further research is needed to clarify the causal relationship. Mechanistically, diabetes and liver cancer share common pathologies, including insulin resistance-related hyperinsulinemia, DNA damage due to increased oxidative stress, and chronic inflammation or lipotoxicity induced by liver fat accumulation.

In recent decades, diabetes and HCC have both become socioeconomic problems with high incidence rates worldwide, in the context of a growing population of individuals with obesity and an increasing prevalence of metabolic syndrome. However, there is no established method for appropriate HCC surveillance in patients with diabetes, and HCC is often detected at an incurable stage. Although some antidiabetic drugs are expected to prevent HCC development, further research on the optimal use of antidiabetic drugs aimed at hepatoprotection is essential. In summary, diabetologists and hepatologists need to work together to study liver diseases in patients with diabetes, and further evidence on the prevention and early detection of HCC occurring in association with diabetes is desired.

 Authors’ contribution

TN and RT contributed to the literature review and manuscript preparation.

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Conflicts of Interest
The authors have no conflicts to disclose.

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Hepatocytes infected with hepatitis C virus change immunological features in the liver microenvironment

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Hepatitis C virus (HCV) infection is remarkably efficient in establishing viral persistence, leading to the development of liver cirrhosis and hepatocellular carcinoma (HCC). Direct-acting antiviral agents (DAAs) are promising HCV therapies to clear the virus. However, recent reports indicate potential increased risk of HCC development among HCV patients with cirrhosis following DAA therapy. CD8⁺ T-cells participate in controlling HCV infection. However, in chronic hepatitis C patients, severe CD4⁺ and CD8⁺ T-cell dysfunctions have been observed. This suggests that HCV may employ mechanisms to counteract or suppress the host T-cell responses. The primary site of viral replication is within hepatocytes where infection can trigger the expression of costimulatory molecules and the secretion of immunoregulatory cytokines. Numerous studies indicate that HCV infection in hepatocytes impairs antiviral host immunity by modulating the expression of immunoregulatory molecules. Hepatocytes expressing whole HCV proteins upregulate the ligands of programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and transforming growth factor β (TGF-β) synthesis compared to those in hepatocytes in the absence of the HCV genome. Importantly, HCV-infected hepatocytes are capable of inducing regulatory CD4⁺ T-cells, releasing exosomes displaying TGF-β on exosome surfaces, and generating follicular regulatory T-cells. Recent studies report that the expression profile of exosome microRNAs provides biomarkers of HCV infection and HCV-related chronic liver diseases. A better understanding of the immunoregulatory mechanisms and identification of biomarkers associated with HCV infection will provide insight into designing vaccine against HCV to bypass HCV-induced immune dysregulation and prevent development of HCV-associated chronic liver diseases.  

Keywords: Hepatitis C; Hepatocellular carcinoma; Immunity; Cell communication; Exosomes

INTRODUCTION

The hepatitis C virus (HCV) is a serious and growing worldwide threat to human health, having already infected approximately 3% of the world’s population (>180 million people).¹ HCV transmission can often be linked to a blood-borne route, such as intravenous drug use or medical procedures. HCV infection is almost invariably associated with viral persistence, leading to development of hepatocellular carcinoma (HCC), as well as, autoimmune diseases such as mixed
cryoglobulinemia. Direct-acting antiviral agents (DAAs) are promising HCV therapies to clear the virus. However, recent reports indicate a potential increased risk of HCC development among HCV-infected patients with cirrhosis following DAA therapy. Unfortunately, development of a vaccine against HCV infection has failed, and no vaccine is currently available.

Since HCV was identified as the causative agent of non-A, non-B hepatitis, the immune responses to HCV infection have been examined in detail. It is notable that immune responses to HCV are significantly impaired. First, the appearance of HCV-specific antibody response is delayed and is detectable on 2–4 months after viral infection. Second, T cell responses to HCV have been demonstrated with multiple antigenic stimulations. Importantly, early and sustained CD4+ and CD8+ T-cell responses are crucial for controlling HCV infection, but the magnitude of T-cell responses is dramatically decreased in chronic hepatitis C patients compared to that in acute hepatitis C patients. This suggests that HCV may employ mechanisms to evade or possibly suppress host T-cell responses. It is important to understand how chronic HCV infection dampens T-cell responses against HCV infection and develop vaccine against HCV.

Numerous studies have reported that HCV actively suppresses the immune response by altering the differentiation of innate immune cells, resulting in the impairment of subsequent robust antiviral adaptive responses. Moreover, CD4+ CD25+ regulatory T-cells (Tregs) have been consistently shown to be expanded in patients with chronic infection. CD4+CD25+ Tregs play a pivotal role in maintaining immune homeostasis and controlling excessive immune responses. The immunoregulatory cytokines, transforming growth factor β (TGF-β) and interleukin (IL)-10, are crucial for the induction and maintenance of Tregs. TGF-β is involved in the generation of inducible Tregs and the maintenance of Treg function. IL-10 is a critical factor for sustaining FoxP3 expression. In addition, these cytokines have been reported to be secreted during HCV infection and have polymorphisms that correlate with HCV clearance.

Molecular biological studies of HCV have shown that it is a positive-stranded RNA virus related to the Flaviviridae family. The viral genome encodes a single polyprotein of approximately 3,000 amino acids (aa) processed by host and viral proteases to form non-structural and structural proteins including a nucleocapsid (core) and two envelope proteins. The primary site of HCV replication is in hepatocytes. HCV life cycle involves multiple steps to generate infectious virus and lipid droplet formation is crucial for viral RNA replication (Fig. 1). Viral tropism seems to be determined by initial interaction of HCV glycoproteins with dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (SIGN) and lymph node-SIGN on the surface of liver endothelial cells and antigen-presenting cells. This interaction is followed by binding to CD81, SR-B1, and/or hepatic sulfate on the cell surface of hepatocytes. Although there is evidence for HCV replication at extrahepatic sites including B-cells, the vast majority of HCV replication and protein expression occur in hepatocytes. Recently, it has been reported that hepatocytes are capable of exerting immunoregulatory function. Notably, HCV-infected hepatocytes interact with immune cells present in the liver microenvironment and suppress host immune responses. In this review article, we discuss the contribution of HCV-infected hepatocytes to regulate host immune responses during HCV infection and the molecular mechanism for their immunoregulatory function.

**IMMUNOLOGICAL FEATURES OF HEPATOCYTES UPON ENCOUNTER WITH VIRAL INFECTION**

Hepatocytes are not traditionally regarded as key players in mounting immune response. However, they have the ability to produce a large variety of cytokines and chemokines.
Thus, in the liver microenvironment, the cellular interaction between lymphocytes and hepatocytes might take place due to the fenestrated structure of hepatic sinusoids, combined with the lack of basal membrane and the low blood flow. Current techniques available for the in vivo analysis of acute HCV infection are limited because the chimpanzee is the only animal susceptible to a natural HCV infection. The in vitro tissue culture of HCV has been used for studying the interaction of infected hepatocytes with immune cells. HCV infection leads to hepatocyte damages that initiate hepatic inflammatory responses by recruiting immune cells (i.e., myeloid and T-cells) at the site of infection. Secretion of immune mediators from infected hepatocytes and immune cells is involved on the activation of hepatic stellate cells (HSCs) and the development of liver fibrosis (Fig. 2).

**Immune mediators produced from infected hepatocytes**

The HCC cell line, Huh7, is established from HCC and commonly used for in vitro studies. Following HCV infection, Huh7 cells are able to produce IL-7, IL-15, and TGF-β, and their expression does not change with IL-1α exposure. Other cytokines and chemokines, such as tumor necrosis factor, IL-1β, regulated upon activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-1 α (MIP-1α), and IL-8, are also produced by hepatocytes, and their productions are increased in response to pro-inflammatory IL-1α activation. In addition, HCV infection is associated with the activation of inflammasomes such as nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, and release of IL-1β secretion. Many of these cytokines and chemokines are important to CD4+ T-cell survival and differentiation. For example, RANTES is CD4+ T-cell recruiting cytokine and contributes to development of Th1 response. While IL-15 enhances Th1 cytokine production and promotes development of an effector phenotype, TGF-β has a negative influence on effector T-cell function and is known to be involved in Treg cell and Th17 cell development. Development of HCV replicon (genotype 1a) as
well as replicating JFH1 virus (genotype 2a) represent a major advancement for studying the interaction of HCV-infected hepatocytes and the host immune system. Successful replication of the viral genome has been superior in the HCC cell line Huh7.5. Huh7.5 cells were generated from HCV-positive parental cell, Huh7, which was cured of HCV using interferon-α treatment. These cells were subsequently receptive to HCV replication such that HCV RNA and proteins could be detected soon after transfection with HCV replicons.

Studies on cytokine analysis using HCC cells have been validated in primary hepatocytes following HCV infection. Programmed cell death protein 1 (PD-1) and PD-1 ligand expression

PD-1 is a receptor for the programmed death-ligand 1 (PD-L1) and PD-L2, and plays a role in dampening host immune responses. Specifically, T-cells activation increased the level of PD-1 expression and engagement of PD-1 with its ligand inhibits their activation, proliferation, and cytokine secretions. Leukocytes, a number of soft tissues, and endothelial cells constitutively express low levels of PD-L1, but induce the expression of PD-L2 under the inflammatory condition. Inflammatory cytokines, including interferon (IFN)-γ, up-regulate PD-L1 and PD-L2 expression on a variety of epithelial cells and leukocytes. The PD-1 pathway is associated with outcome of human disease severity (e.g., autoimmune diseases, cancer). PD-1 ligand expression is seen in a variety of cancers, often correlating with worse cancer outcome. Immunotherapy based on PD-1 blockade has been developed to treat cancer patients.

The pathogenic role of the PD-1 pathway has been demonstrated in the progression of chronic liver diseases by determining the modulation of the inhibitory PD-1 ligands in the liver with chronic inflammation. Chronically damaged livers provide ample opportunity for lymphocyte modulation via PD-1/PD-1 ligand ligation. Indeed primary human hepatocytes as well as Kupffer cells, stellate cells, T-cells, myeloid cells, and liver sinusoidal endothelial cells (LSECs) express PD-L1 and PD-L2. At the messenger RNA (mRNA) level, chronic

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**Figure 2.** Interaction between virus-infected hepatocyte and immune cells. Hepatitis C virus infection and replication in hepatocytes promote the production of cytokines/chemokines leading to recruit immune cells. The excessive cytokines cause hepatic inflammation in the liver and exacerbate tissue damage and liver disease progression. DC, dendritic cell; KC, Kupffer cell; NK, natural killer cell; LSEC, liver sinusoidal endothelial cell.
hepatitis C and autoimmune hepatitis (AIH) patients have increased levels of PD-L1 and PD-L2 mRNA compared to those with normal livers. Multiple studies found that blocking PD-1 and PD-L1 interactions on leukocytes from hepatitis B virus (HBV)- or HCV-infected patients restored T-cell function in vitro.\textsuperscript{33,34}

Cellular location of PD-1 and its ligand expression, has been identified by histologic studies on liver biopsies from chronic hepatitis B, chronic hepatitis C, AIH, and nonalcoholic fatty liver disease (NAFLD) patients as well as individuals with normal liver histology. The presence of the normal control group enabled to differentiate baseline tolerogenic features of the liver from those modulated during chronic liver damage. The increased numbers of CD3\textsuperscript{+} T-cells were detected in chronic hepatitis B, chronic hepatitis C, and AIH livers and significant portions of intrahepatic lymphocytes from these patient groups expressed PD-1. LSECs, Kupffer cells, and intrahepatic leukocytes expressed PD-L1 and PD-L2 while hepatocytes also expressed PD-L1 and PD-L2 under inflammation. These studies confirm that PD-L1 and PD-L2 expression on parenchymal and non-parenchymal cells can deliver a negative signal to T-cells, dampening their responses. Moreover, the necroinflammatory levels associated with chronic hepatitis B, chronic hepatitis C, and AIH were correlated with increased PD-L1 and PD-L2 on leukocytes, Kupffer cells, and LSECs. However, early-stage NAFLD patients did not demonstrate significant increases in CD3\textsuperscript{+} lymphocyte infiltrates, PD-1 or PD-L1 and PD-L2 expression, suggesting that inflammation rather than liver damage itself leads to the expression of PD-1 and PD-1 ligands.

**Induction of Treg driven by TGF-β secreted from HCV-infected hepatocytes**

Impaired antiviral CD8\textsuperscript{+} and CD4\textsuperscript{+} Th1 T-cell responses are associated with persistence of HCV infection.\textsuperscript{35} Although failure of T-cell responses might occur as a result of mutation in viral antigens,\textsuperscript{36,37} and upregulation of negative costimulatory PD-1 and CTLA-4 pathways,\textsuperscript{38,39} HCV infection generates a direct mechanism to generate CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Tregs to inhibit T-cell responses. Notably, an increase in the number and functionality of Tregs has been detected in chronic HCV patients as compared to those with resolved infection.\textsuperscript{40,41} The increased frequency of Tregs observed in chronic HCV patients might arise from expansion of thymic-derived natural Tregs or from de novo induction from naive T-cells. The mechanism underlying induction of Tregs during HCV infection remains unclear.

Notably, HCV protein expression within hepatocytes alters the function of CD4\textsuperscript{+} T-cells and could contribute to development of Tregs.\textsuperscript{42} By using an HCV whole protein-expressing hepatoma line (Huh7.5-FL), studies have been conducted to examine contribution of infected hepatocytes on CD4\textsuperscript{+} T-cell dysfunction. CD4\textsuperscript{+} T-cell responsiveness, as measured by IFN-γ production, was diminished in co-culture with Huh7.5-FL compared to controls. Importantly, CD4\textsuperscript{+} T-cells in contact with Huh7.5-FL adopted a Treg phenotype (CD25\textsuperscript{+}FoxP3\textsuperscript{+}CTLA-4\textsuperscript{+}LAP\textsuperscript{+}) and developed the ability to suppress effector T-cell proliferation. The role of hepatocytes in Treg development was clarified by finding that Huh7.5-FL produced more TGF-β than control hepatocytes. Moreover, intracellular expression of an HCV core is known to enhance TGF-β1 mRNA production by the hepatoma cell line HepG2.\textsuperscript{43,44} These provide evidence that the site of HCV infection (i.e., hepatocytes) plays a pivotal role in impairing the antiviral T-cell response by the induction of Tregs via TGF-β production.

**CELLULAR CROSSTALK VIA EXOSOMES RELEASED BY HCV-INFECTED HEPATOCYTES**

Cells exchange information through release of soluble factors or by direct interaction. Several reports demonstrate that cells can also communicate by circular membrane fragments called extracellular vesicles (EVs).\textsuperscript{45} Normal or diseased cells release different types of EVs, including microvesicles (MVs) and exosomes, depending on their cellular origin. Exosomes (40–100 nm) are formed by the fusion between multi-vesicular bodies and the plasma membrane, while MVs (100–2,000 nm) bud directly from the plasma membrane. Exosomes have been shown to provide a means of intercellular communication as contributing factors in the development of several diseases by the spread of proteins, mRNAs, and microRNAs (miRNAs).\textsuperscript{46} During virus infection, exosomes released from virus-infected cells contain viral proteins, viral RNAs, and certain specific miRNAs that are able to spread the infection and alter the cellular response in uninfected target cells during the immune response and pathogenesis.

Exosomes secreted from HCV-infected hepatocytes play a critical role in promoting intercellular crosstalk with liver non-
parenchymal cells. HCV infection may directly activate a signaling network in hepatocytes, promoting release of immunoregulatory molecules packaged into exosomes, leading to intercellular communication inducing for activation of fibrotic macrophages and LSEC (Fig. 3). Recently, accumulating evidence demonstrates that exosomes and exosomal miRNAs from HCV-infected hepatocytes lead to polarization and differentiation of macrophages and mediate pro-fibrotic responses in HSC and T follicular regulatory (Tfr) cells expansion. This suggests that development of liver disease involves intercellular communication during HCV infection. Interestingly, some studies have reported increased release of specific miRNAs, such as miR-122 in HCV infection. Recently, potential cellular and molecular mechanisms of HCV-mediated secretions of exosome and exosomal miRNAs have been elucidated.

**Exosomes containing immunoregulatory molecules**

Numerous studies have been conducted to identify contents of exosomes secreted from HCV-infected hepatocytes and define their biological function. Interestingly, HCV exosomes play a role in regulating host immune responses and facilitating development of persistent HCV infections and chronic liver diseases. HCV-dependent elevated reactive oxygen species (ROS) levels and induction of autophagy are related to exosomes derived from the endosomal pathway. Toll-like receptor (TLR) 7 and TLR8 are present in intracellular vesicles from HCV-infected hepatocytes and macrophages. TLR is a type of pattern-recognition receptor in the immune system recognizing pathogen-associated molecular patterns (PAMPs) and exerts a broad spectrum of innate immunity. Exosomes containing single-strand HCV RNA have been shown to affect differentiation of monocytes to fibrogenic macrophages in a TLR7/8-dependent manner. TLR3 activation was reduced under influence of viral dsRNA contained in exosomes secreted from HCV-positive cells, showing a novel mechanism to evade the host immune response in virus persistence.

Moreover, HCV exosomes isolated from infected hepatocytes contain TGF-β at the surface of exosomes. TGF-β is important for induction and expansion of Tfr cells, a subset of Tregs. Increased Treg responses are a prominent feature in

![Figure 3. Schematic diagram of HCV exosomes. Exosomes released by HCV-infected hepatocytes promote intercellular crosstalk with Mϕ and LSEC leading to stellate cell activation. HCV, hepatitis C virus; LSEC, liver sinusoidal endothelial cells; TGF-β, transforming growth factor β; miRNA, microRNA; HSC, hepatic stellate cell.](https://doi.org/10.3350/cmh.2022.0032)
HCV infection such that Tregs are increased in both number and function in chronic hepatitis C patients and are positively correlated with viral load. Furthermore, abundant Tregs are found in the livers of chronic hepatitis C patients. Recently Tfr cells have been identified for functional regulation of germinal center (GC) responses by limiting Tfh cells and B cells. Tfrs are reported to be increased in the circulation of chronic hepatitis C patients. Tfrs are identified by the expression of follicular markers CXCR5 and PD-1 and regulatory markers CD25 and Foxp3. This allows Tfrs to co-migrate with Tfh to control GC responses. Interestingly, lymphoid follicles, containing T- and B-cells, are commonly observed in the livers of HCV-infected patients and exhibit signs of GC-like architecture. Recent studies have identified the presence of Tfrs within the livers of HCV-infected patients.

Exposure of CD4+ T-cells to TGF-β-containing exosomes from HCV-infected hepatocytes led to a significant increase in Tfrs. This study has been done by culturing exosomes isolated from HCV-infected primary hepatocytes with pre-activated CD4+ T-cells. Moreover, depletion of CD14+ monocytes prior to coculture of infected hepatocytes with PBMCs did not affect the ability of infected hepatoma cells to drive Tfr expansion but monocytes are not required for expansion of Tfr cells. Importantly, expansion of Tfr cell is accompanied by acquisition of an enhanced regulatory phenotype and leads to the functional suppression of Tfh cells. Increases in Tfr responses are driven by a novel pathway involving the release of TGF-β-containing exosomes from HCV-infected hepatocytes. These findings highlight the accumulation of Tfrs in the livers of HCV-infected patients, potentially inhibiting protective Tfh and B-cell responses at the site of infection, and contributing to viral persistence.

**Exosomes containing miRNAs**

Several studies report the expression and biological activity of various miRNAs in HCV infection-associated exosomes. In the exosomes of HCC patients, miR-21-5p, miR-10b-5p, miR-221-3p, and miR-223-3p were significantly upregulated compared to the non-HCC individuals. miR-19a and miR-192 from exosomes secreted from HCV-infected hepatocytes were internalized into HSCs and induced HSC activation by triggeringSTAT3-mediated TGF-β signaling. HCV-induced exosomal miR-122/let-7b/miR-206 induced activation of B-cells associated with mixed cryoglobulinemia. A link between the runt-related transcription factor1 (RUNX1)/RUNX1 overlapping RNA (RUNXOR) and the STAT3/miR-124 pathway regulated differentiation of myeloid-derived suppressor cells (MDSCs) during chronic HCV infection, and expression of miR-124 was negatively correlated with expression of STAT3. In addition, a pilot study on expression profiles of exosomal miRNAs in HCV-infected patients has identified various miRNAs related to other diseases.

**Molecular mechanism of exosome release from infected hepatocytes**

The exosome plays a critical role in mediating the cellular communication. Syntenin has been reported to be involved on the secretion of E2 via exosomes. E2 is a viral envelope glycoprotein that forms a heterodimer and mediates viral entry. The release of MVs or exosomes can be stimulated by

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**Table 1. Candidate biomarkers of HCV exosomal miRNAs**

<table>
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<tr>
<th>Biomarker</th>
<th>Responses of each markers in HCV</th>
<th>Reference</th>
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<tr>
<td>miR-21-5p, miR-10b-5p, miR-221-3p, miR-223-3p</td>
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<td>miR-19a</td>
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<td>miR-627-5p, miR-221</td>
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<td>miR-155</td>
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<tr>
<td>miR-199a</td>
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</table>

HCV, hepatitis C virus; miRNA, microRNA.
stress signals, including DNA damage, intracellular calcium, and extracellular adenosine triphosphate (ATP). Exosome release can occur by an ESCRT-dependent or ESCRT-independent pathway. Moreover, exosome release is induced by extracellular ATP that is associated with purinergic receptor activation.

Pannexin 1 (Panx1) is a transmembrane channel that mediates ATP release. Panx1 is activated by the stretch of the plasma membrane during changes in osmolality or mechanical injuries or by proteolysis via caspase-3 and -7 during early apoptosis. The ATP released by Panx1 activation can bind to the purinergic receptor, leading to a calcium influx. Expression of Panx-1 and purinergic receptor was increased in HCV-infected hepatocytes. However, participation of Panx1 pathway-mediated exosome release in viral infection has not been well elucidated. Our studies demonstrate that secretions of exosomes and specific miRNAs are associated with the Panx1/purinergic receptor pathway in HCV-infected hepatocytes. Notably, Panx1 inhibitors prevented release of exosomes from HCV-infected hepatocytes.

DEVELOPMENT OF FUTURE THERAPEUTICS TO TREAT CHRONIC LIVER DISEASE

Therapeutic interventions to develop drugs for halting the liver disease progression and vaccine for preventing HCV infection have met with limited success. It is vital to understand the pathogenesis of HCV infection and the mechanism of virus-induced immune suppression leading to the establishment of persistent infection. The studies described in this review article provide novel and important information on the role of HCV-infected hepatocytes in the pathogenesis of HCV infection and inhibition of T-cell function. Results of these studies contribute to advance the understanding of impaired T-cell responses via interactions between hepatocyte and T-cell. Thus these data should stimulate development of novel vaccine strategies for this important human pathogen.

Furthermore, miRNA-containing exosomes have been reported as biomarkers for diagnosis of HCV. Table 1 summarizes miRNAs identified as biomarkers associated with HCV infection. Exosomes containing miR-19a and miR-192 were observed in the serum of HCV patients and presented as a new marker. An increase of exosome miR-885-5p and miR-365 but a decrease of exosome miR-627-5 and miR-221 showed characteristic of HCV-infection among other miRNAs. Expression of exosome miR-155 was reduced after rituximab treatment in HCV patients. In particular, serum miR-122 and miR-199a are potential biomarkers reflecting therapeutic efficacy against HCV infection. Potential mechanisms of HCV anti-viral therapy involve therapeutic agents directly acting on the virus, IFN-dependent/independent therapeutics, and host-targeting antivirals (HTAs). miR-122 increases viral replication by directly binding to two conserved flanking regions of the 5’ UTR of HCV RNA and acts as HTA against HCV replication. Miravisen, miR-122 antisense blocker, has been developed as a latest HTA. In addition, treatment of syntenin, a protein involved in the exosome secretion pathway, has recently been introduced, but like the above-mentioned treatment, there are few reports of its clinical test results yet. Nevertheless, these studies are important for developing therapeutics to target HCV-infected hepatocytes and prevent development of HCV-associated chronic liver diseases.

CONCLUSION

In summary, HCV-infected hepatocytes play a pivotal role in changing immunological features in the liver microenvironment. Through cellular and molecular mechanisms, HCV-infected hepatocytes dampen intrahepatic T-cell responses directly via increased expression of PD-L1 or indirectly by releasing immunoregulatory molecules such as TGF-β. Future studies are needed to develop immune-based therapeutics to treat chronic liver diseases associated with HCV infection. In addition, the markers in the various immunological mechanisms presented in this review can be used in future research on immune-based therapeutics.

Authors’ contribution
SJP contributed to manuscript research and writing. YSH contributed to conceptualization of review article and critical review/editing of the manuscript.

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Conflicts of Interest

The authors have no conflicts to disclose.

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Pathogenesis and treatment of non-alcoholic steatohepatitis and its fibrosis

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The initial presentation of non-alcoholic steatohepatitis (NASH) is hepatic steatosis. The dysfunction of lipid metabolism within hepatocytes caused by genetic factors, diet, and insulin resistance causes lipid accumulation. Lipotoxicity, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress would further contribute to hepatocyte injury and death, leading to inflammation and immune dysfunction in the liver. During the healing process, the accumulation of an excessive amount of fibrosis might occur while healing. During the development of NASH and liver fibrosis, the gut-liver axis, adipose-liver axis, and renin-angiotensin system (RAS) may be dysregulated and impaired. Translocation of bacteria or its end-products entering the liver could activate hepatocytes, Kupffer cells, and hepatic stellate cells, exacerbating hepatic steatosis, inflammation, and fibrosis. Bile acids regulate glucose and lipid metabolism through Farnesoid X receptors in the liver and intestine. Increased adipose tissue-derived non-esterified fatty acids would aggravate hepatic steatosis. Increased leptin also plays a role in hepatic fibrogenesis, and decreased adiponectin may contribute to hepatic insulin resistance. Moreover, dysregulation of peroxisome proliferator-activated receptors in the liver, adipose, and muscle tissues may impair lipid metabolism. In addition, the RAS may contribute to hepatic fatty acid metabolism, inflammation, and fibrosis. The treatment includes lifestyle modification, pharmacological therapy, and non-pharmacological therapy. Currently, weight reduction by lifestyle modification or surgery is the most effective therapy. However, vitamin E, pioglitazone, and obeticholic acid have also been suggested. In this review, we will introduce some new clinical trials and experimental therapies for the treatment of NASH and related fibrosis. (Clin Mol Hepatol 2023;29:77-98)

Keywords: Liver fibrosis; Steatohepatitis; Fatty liver
INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently the most prevalent type of liver disease worldwide. NAFLD is a wide hepatic spectrum, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which leads to progressive fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Fat accumulation in hepatocytes sensitizes hepatocytes to injury, leading to cell death, inflammatory cells recruitment, and activation of hepatic stellate cells (HSCs). The pathogenesis of NASH and its fibrosis has been broadly investigated for decades, and the development and progression of NASH and liver fibrosis involves complex interplay of numerous determinants. Understanding of the pathogenesis of NASH and liver fibrosis is important for the diagnosis and development of treatment. Although new drugs have been developed to target liver inflammation and fibrosis in NASH, only a minority of patients achieve treatment response. Thus, there is still an urgent need to develop new therapeutic agents for NASH.

PATHOGENESIS OF NASH

Development of hepatic steatosis

Diet

High-fat diet can result in hepatic steatosis in humans. Liver fat increased by 35% in overweight non-diabetic women after a 2-week isocaloric high-fat diet (56% total energy from fat). A single energy-dense, high-fat meal induced net lipid accumulation in the liver of healthy subjects. Moreover, palm oil administration in lean, healthy individuals decreased whole-body, hepatic, and adipose tissue insulin sensitivity by 25%, 15%, and 34%, respectively; increased hepatic TG and ATP content by 35% and 16%, respectively; increased hepatic gluconeogenesis by 70%; and decreased glycolysis by 20%. In young Finnish adults, serum fatty acid saturation independently predicted the 10-year risk for fatty liver and omega-6 (ω6) fatty acids inversely associated with fatty liver. A long-term hypercaloric diet, rich in saturated fatty acid (SFA), showed a marked increase in liver fat content by 50%, and ω6 polyunsaturated fatty acids (PUFAs) decreased fatty liver in overweight humans. However, a lipidomic analysis showed that the n-6:n-3 free fatty acids (FFAs) ratio increased in NASH livers as compared to normal livers. These studies suggested that hypercaloric diet, especially high in fat and sugar, contribute to the development of fatty liver; SFA and fructose are more detrimental, but the role of the ω6/ω3 fat ratio also remains controversial.

Physical inactivity

NAFLD patients have low level of physical activity compared to normal controls. Gerber et al. showed that the average physical activity, counted by an accelerometer of NAFLD subjects, was about 28.7 counts/minute/day. In an Asian group, prolonged sitting time and decreased physical activity level were found to positively associated with the prevalence of NAFLD, and these associations were also observed in subjects with body mass index <23 kg/m². However, the detail mechanism of sedentary behavior or low physical activity leading to fatty liver remains unclear. Lower expenditure of energy or lower skeletal muscle mass might explain a possible connection between sedentary behavior and NAFLD.

Abbreviations:

- ACC, acetyl-coenzyme A carboxylase
- AMPK, AMP-activated protein kinase
- ANG, angiotensin
- ASK1, apoptosis signaling kinase 1
- DGAT2, diacylglycerol O-acyltransferase 2
- DJBL, duodenal-Jejunal bypass liner
- DMR, duodenal mucosal resurfacing
- DNL, de novo lipogenesis
- ER, endoplasmic reticulum
- ESG, endoscopic sleeve gastrectomy
- FFA, free fatty acid
- FGF, fibroblast growth factor
- FMT, fecal microbiota transplantation
- FXR, farnesoid X receptor
- GCKR, glucokinase regulatory protein
- GLP-1, glucagon-like peptide-1
- HCC, hepatocellular carcinoma
- Hh, hedgehog
- HSP, hepatic stellate cell
- IGB, intragastric balloons
- IL, interleukin
- IRE1, inositol-requiring enzyme 1
- IRGM, immunity-related GTPase M1
- KC, Kupffer cell
- LSG, laparoscopic sleeve gastrectomy
- MBOAT7, membrane-bound O-acyltransferase domain-containing protein 7
- miRNA, microRNA
- NAFLD, non-alcoholic fatty liver disease
- NAS, NAFLD activity score
- NASH, non-alcoholic steatohepatitis
- NEFA, non-esterified fatty acid
- OCA, obesticholic acid
- PAI-1, plasminogen activator inhibitor-1
- PERK, protein kinase R-like ER kinase
- PNPLA3, patatin-like phospholipase domain-containing 3
- PPAR, proliferator-activated receptor
- PRR, (pro)renin receptor
- PUFAs, polyunsaturated fatty acids
- RAS, renin-angiotensin system
- RCT, randomized controlled trial
- SCD-1, stearoyl-coenzyme A desaturase 1
- SFA, saturated fatty acid
- SGLT2, sodium-glucose cotransporter 2
- SREBP1c, sterol regulatory element-binding protein 1c
- TG, triglyceride
- TNF, tumor necrosis factor
- UPR, unfolded protein response
- VLDL, very-low-density lipoprotein
- XBP-1, X-box-binding protein 1
- ω6, omega-6
and NAFLD.

**Insulin resistance**

NAFLD is strongly associated with reduced whole body insulin sensitivity, as well as increased hepatic and adipose tissue insulin resistance. Insulin resistance can lead to hepatic fat accumulation by increasing FFA delivery to the liver, increasing de novo lipogenesis (DNL), and decreasing hepatic fatty acid oxidation. A landmark study performed by Donnelly et al. demonstrated that in NAFLD patients, about 59% liver triacylglycerol arose from NEFAs, 26.1% from DNL, and 14.9% from the diet, and that the liver demonstrated reciprocal use of adipose and dietary fatty acids. DNL was elevated in the fasting state without diurnal variation. Insulin resistance can impair the insulin suppression of lipolysis of peripheral adipose tissues, leading to increased delivery of FFAs to the liver. Insulin can stimulate sterol receptor binding protein 1-c (SREBP1c), increasing DNL in the liver. Chronic hyperinsulinemia results in the cytoplasmic localization and inactivation of Foxa2 phosphorylation in hepatocytes, thereby promoting lipid accumulation and insulin resistance in the liver.

**Genetic factors**

There are several gene variants associated with NAFLD and NASH. The first fatty liver gene identified by Romeo et al. is patatin-like phospholipase domain-containing 3 (PNPLA3). The single nucleotide polymorphism (SNP) rs738409 causes the missense sequence variation I148M, impairing the phospholipase activity and increasing hepatic fat content. Glucokinase regulatory protein (GCKR) can regulate hepatic glucose uptake and hepatic glucokinase activity, and the intronic SNP rs780094 is associated with hepatic lipid content. The SNP rs1260326 (C>T; P446L), GCKRP446L can decrease the inhibition of glucokinase, leading to increased glycolytic flux to hepatocytes, then hepatic steatosis. The rs1260326 (G>A; E167K) variant, transmembrane 6 superfamily 2 (TM6SF2), was associated with increased hepatic TG content. The inhibition of TM6SF2 in hepatocytes reduced the secretion of very-low-density lipoprotein (VLDL), leading to the retention of TGs. In a Taiwanese population, a variant in the immunity-related GTPase M (IRGM) gene (rs10065172 TT genotype) independently increased the odds ratio of NAFLD by 2.04 by altering hepatic lipid metabolism through the autophagy pathway. Similarly, the IRGM rs10065172 variant increased the risk for hepatic steatosis, but not for liver inflammation or fibrosis, in obese Italian children. Recently, the mechanisms underlying metabolic and genetic components of NAFLD were found to be fundamentally different in patients. The metabolic component is characterized by hepatic oversupply of sugars and lipids, while the genetic component is characterized by impaired hepatic mitochondrial function, reducing the liver’s ability to metabolize these substrates.

**Epigenetic factor**

Using an epigenome-wide association study in peripheral blood cells, 22 CpGs were found to be associated with hepatic fat in European participants; 19 CpGs were annotated to 18 unique genes upregulated in the liver, including DHCR24, SLC43A1, CPT1A, SREBF1, SC4MOL, and SLC9A3R1. Some alternations of intrahepatic microRNA (miRNA) have been associated with hepatic steatosis. The serum levels of miR-122 and miR-192 were upregulated in patients with simple steatosis compared to normal controls. The administration of exosomes transfected with obesity-associated miRNA induced hepatic steatosis in lean mice. miR-122 inhibition in normal mice caused increased hepatic fatty acid oxidation. Decreased miR-122-5p in the human liver was associated with impaired fatty acid usage. However, the deletion of mouse miR-122 resulted in hepatosteatosis, inflammation, and the development of tumors. The expression of miR-34 was elevated in NAFLD patients. miR-34a down-regulated autophagy in hepatocytes by targeting ATG4B and Rab-8B and suppressed mitochondrial biogenesis, leading to lipids accumulation in the liver.

**Lipotoxicity**

**Endoplasmic reticulum (ER) stress**

The ER is responsible for protein folding, and the accumulation of misfolded or unfolded proteins leads to stress and the activation of the unfolded protein response (UPR). There are three sensor proteins that activate UPR, namely the inositol-requiring enzyme 1 (IRE1), the protein kinase R (double-stranded RNA-activated protein kinase)-like ER kinases (PERK), and the activating transcription factor 6. The UPR can cause inflammation, inflammasome activation, and death of hepatocytes. Patients with NASH have been shown to be specifically associated with failure to generate X-box-binding
protein 1 (XBP-1) protein and activation of JNK. Palmitate can induce the ER stress response, as demonstrated by the increase in C/EBP homologous protein (CHOP) expression, eIF2-alpha phosphorylation, XBP-1 splicing, and JNK activation with increased expression of the BH3-only proteins PUMA and Bim. Perturbation of membrane lipid composition could promote IRE1 and PERK activation, suggesting a lipid-sensing mechanism for ER sensors to activate the UPR. NFATc1 drives hepatocyte damage and inflammation through activation of the PERK-CHOP.

Mitochondrial dysfunction
Increased hepatic fat would increase hepatic fat oxidation with increased mitochondrial respiration, however, decreased efficiency of respiratory chain complexes with greater mitochondrial uncoupling and leaking activity was found in patients with NAFLD. Chronic mitochondrial dysfunction in the state of lipid overload led to excessive leakage of electrons from mitochondrial respiratory complexes, leading to oxidative stress. Voltage-dependent anion channel acted as an early sensor of lipid toxicity, and its glycerol synthase kinase 3-mediated phosphorylation status controlled outer mitochondrial membrane permeabilization in hepatocytes with fat accumulation. Exposure of hepatocytes to saturated FFA caused mitochondrial depolarization, cytochrome c release, and increased ROS production. Furthermore, intake of SFAs can affect the composition of mitochondrial membrane and decrease the efficiency of the respiratory transport chain, resulting in increased oxidative stress and chronic liver injury. Peng et al. found that hepatic cardiolipin and ubiquinone accumulated in NAFL and the levels of acylcarnitine increased with NASH, and proposed that increased levels of cardiolipin and ubiquinone may help to preserve mitochondrial function in early NAFLD; however, mitochondrial function eventually fails with the progression of NASH, leading to increased acylcarnitine. Moreover, SFAs increased ceramide synthesis in hepatocytes, which correlated with hepatocyte death via mitochondrial failure.

Lysosomal dysfunction
It has been shown that hepatic activity of lysosomal acid lipase and lysosomal acidification, which are markers of lysosomal dysfunction, are decreased in patients with NAFLD. Both steatotic- and asparagine-treated hepatocytes showed reduced lysosomal acidity and retention of lysosomal calcium. FFA resulted in Bax translocation to lysosomes and lysosomal destabilization with the release of cathepsin B into the cytosol, leading to nuclear factor kappa B-dependent tumor necrosis factor alpha expression and apoptosis. Lyso- somal permeabilization and cathepsin B redistribution into the cytoplasm occurred several hours prior to mitochondrial dysfunction. Furthermore, autophagy could sequester intracellular proteins and organelles in double-membrane vesicles (autophagosomes) to lysosomes for degradation. Autophagy in the regulation of intracellular lipid stores is called macrolipophagy. Toxic fatty acids inhibited autophagic flux with reduction in lipophagy, which could lead to cell injury.

Oxidative stress and apoptosis
The main mechanisms of fatty acid-induced damage are oxidative stress and increased pro-inflammatory cytokines. These insults from the ER stress, mitochondrial dysfunction, and oxidative stress in the hepatocytes after lipid accumulation could cause lipotoxicity, leading to apoptosis, necroptosis, or pyroptosis. Saturated FFAs can also induce apoptosis through intrinsic and extrinsic pathways. The oxidative stress and ER stress induced by accumulated fatty acids can activate CHOP and JNK, and then upregulate Bim, Bax, and Bak, leading to the release of cytochrome C and caspase 9-associated apoptosis. In addition, death receptor pathways, including TRAIL/TRAIL receptor, tumor necrosis factor-α (TNFα)/TNF receptor 1 (TNFR1), and Fas ligand/Fas, were noted to be activated by FFAs on hepatocytes.

DEVELOPMENT OF NASH FIBROSIS
Liver fibrosis is the most important risk factor for liver cancer in patients with NAFLD and decompensated cirrhosis. In patients with NAFLD, age and comorbidities, such as hypertension, overweighted, and diabetes mellitus, are risk factors for progression of fibrosis. Lipotoxic damage in hepatocytes would release cytokines and chemokines, and then activate innate and adaptive immune cells, including macrophages, dendritic cells, lymphocytes, and neutrophils, leading to an inflammation cascade. Damaged hepatocytes also release extracellular vesicles containing exosomes, microparticles, and apoptotic bodies. These vesicles, containing signaling proteins, sonic hedgehog (Hh), lipids, mRNAs, non-coding RNAs, and DNA, can in-
duce inflammation, fibrosis by activating non-parenchymal cells, and recruitment of immune cells. Meanwhile, apoptotic bodies can also be engulfed by stellate cells and subsequently induce HSC activation, which increases the expression of α-smooth muscle actin, transforming growth factor β (TGFβ), and collagen type I. Moreover, the Hh pathway was not only activated in hepatocytes, leading to macrophage recruitment and progression of inflammation, but it also induced epithelial-to-mesenchymal transitions in ductular-type progenitors. Cholangiocytes and natural killer T cells also activated Hh-osteopontin pathway and promoted fibrogenic responses of HSCs in NASH.

Toxic fatty acids were able to directly affect Kupffer cells (KCs) and HSCs, which may contribute to the activation of inflammation and fibrosis. Palmitic acids activated toll-like receptor (TLR) 2 and TLR4 in macrophages with the induction of inflammatory signaling. KCs exhibited a pro-inflammatory response with elevated levels of TNFα, interleukin (IL)-6, and IL-1β after treatment by palmitic acids. Palmitate induced ER stress and actin stress fiber formation in activated HSCs. Oleate induced the inflammatory signal and decreased cytoskeleton proteins in activated HSCs. Free cholesterol was increased in patients with NAFLD, and the accumulation of free cholesterol in HSCs sensitized these cells to TGFβ-induced activation, leading to exaggerated liver fibrosis in NASH.

Insulin exerts profibrogenic activity. Insulin itself induces HSC mitogenesis and collagen synthesis. However, insulin enhances the expression of smooth muscle actin-α in quiescent, but not in activated HSC through the PI3K/Akt-p70S6K pathway.

HSCs express PNPLA3 and membrane-bound O-acyltransferase domain-containing protein 7 (MBOAT7). Increased PNPLA3 expression reduces lipid droplet content in HSCs. Autophagy promotes loss of lipids in HSCs to provide energy for HSC activation. PNPLA3 can interfere with retinol production and release of HSCs by affecting the retinyl-palmitate lipase activity, which may promote fibrosis progression. The MBOAT7 rs641738 T allele was associated with lower protein expression in the liver, and changes in plasma phosphatidylinositol species were consistent with decreased MBOAT7 function. Hepatocyte-specific knockout of Mboat7 increased hepatic fibrosis with increased total lysophosphatidylinositol levels, which could promote the initiation of HSC activation by stimulating G-protein receptor 5z. TM6SF2 was associated with higher risk of advanced fibrosis in NAFLD patients. Furthermore, the gene encoding for the hepatic hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) regulated hepatic phospholipids and chronic liver injury in NAFLD patients. The HSD17B13 rs72613567 variant led to the loss of enzyme function, contributing to reduced inflammation and fibrosis in the liver. In addition, the HSD17B13 rs72613567 variant affected retinol metabolism by reducing the activity of retinyl-palmitate lipase, mediating antifibrotic and anti-inflammation effects.

Hypomethylation or hypermethylation of genes involved in the wound-healing process in NAFLD could be used to distinguish between patients with mild fibrosis from those with severe fibrosis in NAFLD. Hypomethylation at specific CpGs within TGFβ1 and PDGF, and hypomethylation at specific CpGs within peroxisome proliferator-activated receptor (PPAR) α and PPARδ in patients with mild fibrosis, were found.

ORGAN-ORGAN INTERACTION LEADING TO PROGRESSION OF NASH AND ITS FIBROSIS

Gut-liver axis

Compared with healthy adults, patients with NAFLD had a higher proportion of Firmicutes in the intestine, and the relative numbers of Bacteroidetes, Enterobacteriaceae, and Ruminococcaceae families were reduced. Dysbiosis may disturb gut barriers, and bacteria and its products from the gut, such as endotoxin and cytokines, that promote inflammation, could enter the liver through blood, and activate the immune system to recognize bacteria and its products effectively improved the severity of steatohepatitis. Bacterial products and translocated lipopolysaccharide stimulated the hepatic innate immune system through TLR4 signaling, predominantly on HSCs and KCs. TLR4-mediated stimulation of HSCs led to HSC activation and KC activation. In turn, KCs produced TGFβ, stimu-
lating fibrogenesis, and the proinflammatory cytokines, propagating hepatic inflammation. KCs also produced reactive oxygen species, leading to the generation of other reactive nitrogen species and local tissue damage. Mice fed with high-fat diet for only 1 week underwent a diet-induced dysbiosis, driving the damage on gut vascular barrier and causing bacterial translocation into the liver. However, only 42.1% of patients with steatohepatitis had elevated endotoxin levels and 39.1% of fatty liver patients had increased intestinal permeability. Therefore, bacterial translocation due to gut barrier impairment may play a partial role in the development and progression of NASH and its fibrosis.

Some metabolites in the blood and feces have been found to rely on bacterial synthesis, including choline and choline-related metabolites, bile acids, short-chain fatty acids (SCFAs), and ethanol, which may contribute to the pathogenesis of fatty liver. In animal experiments, the gut microbiota of mice fed with high-fat diet could convert choline into trimethylamine, reduce the bioavailability of choline, and produce a phenomenon similar to choline-deficient diet, leading to decreased excretion of VLDL from liver cells and increased liver fat accumulation. Intestinal dysbiosis would increase the deoxycholic acid/mucodeoxycholic acid ratio, reduce the activation of farnesoid X receptor (FXR) signaling in the liver, reduce insulin sensitivity, increase glycogen and lipogenesis, and reduce fatty acid oxidation in the liver. At the same time, gut dysbiosis also inhibited FXR, reduced the secretion of fibroblast growth factor (FGF) 15/19, leading to fatty liver. SCFAs, such as acetate, propionate, and butyrate, are the products of bacterial fermentation of carbohydrate in the gut. SCFAs in the intestine enter the liver through the portal vein, and acetate and propionate are precursors for fatty acid synthesis and gluconeogenesis, promoting liver fat accumulation. Furthermore, SCFAs bind to G protein-coupled receptors of intestinal neuroendocrine L cells to secrete peptide YY and glucagon-like peptide-1 (GLP-1), which promote nutrient absorption and liver fat generation. Butyrate may activate the AMP-activated protein kinase (AMPK) pathway in the liver, leading to the inhibition of oxidative stress and inflammation, upregulation of fatty acid oxidation, downregulation of fat synthesis genes, and reduced hepatosteatosis. Interestingly, patients with NAFLD and severe hepatic fibrosis had more acetate in the stool, while those with milder severity of NAFLD had more SCFAs in butyrate and propionate. Moreover, the concentration of ethanol in the blood of patients with NAFLD increased, and the bacteria Proteobacteria, which could produce ethanol, also tended to increase in patients with steatohepatitis. Ethanol destroys the tight binding protein of the intestinal wall, increases the intestinal permeability, and increases the endotoxin entering blood and the liver, leading to liver inflammation.

Adipose tissue-liver axis

Adipose tissues secrete adiponectin, leptin, and some proinflammatory cytokines, such as IL-6 and TNFα, which would influence the liver. Adiponectin binds to adiponectin receptors 1 and 2, respectively activates AMPK and PPAR-alpha pathways in the liver, and stimulate glucose use and fatty acid oxidation. Adiponectin also increases carnitine palmitoyltransferase I activity, enhances hepatic fatty acid oxidation, and decreases the activities of acetyl-CoA carboxylase and fatty acid synthase. However, adiponectin produced mainly from white adipose tissue is decreased in NASH patients. When obesity develops, leptin secreted from white fatty tissue is increased to inhibit appetite and increase fatty acid oxidation. However, in obese individuals, leptin resistance develops, and the increased leptin would exert proinflammatory activity. The serum leptin levels are positively associated with the severity of liver inflammation and fibrosis. Leptin augments the endothelin-1-induced contraction of HSCs. Adipocytes also secrete TNFα, which can increase insulin resistance and have pro-inflammatory effects. TNFα increased the gene expression of Mcp1, Tgfβ1, and Timp1 in hepatocytes, and the Tnf knockout improved glucose tolerance and significantly reduced the prevalence of hepatic steatosis and fibrosis in mice, indicating that TNFα plays a role in the development and progression of NASH. IL-6 can be secreted from adipocytes, which can then increase the macrophage infiltration of adipose tissue. IL-6 infusion induces hepatic insulin resistance through increased adipose tissue lipolysis. These data suggest that IL-6 is involved in the pathogenesis of hepatic insulin resistance.

Renin-angiotensin system (RAS)

Hypertensive patients with biopsy-proven NAFLD on baseline RAS blockers had less advanced hepatic fibrosis. Recently, a large retrospective study showed that angiotensin-converting enzyme inhibitors/angiotensin receptor blockers
were associated with lower risk of hepatocellular carcinoma and cirrhotic complications in patients with NAFLD. These data suggested a beneficial effect of RAS blockers in NAFLD. Transgenic hypertensive rats overexpressing the mouse renin gene with elevated levels of tissue angiotensin II developed hepatic steatosis, inflammation, and fibrosis. The mice lacking the renin gene fed with high-fat diet had decreased liver fat. Alikiren, a direct renin inhibitor, reduced hepatic steatosis in high-fat diet-fed mice and fibrosis in mice fed with methionine-choline-deficient diet. When renin or prorenin binds to the (pro)renin receptor (PRR), in addition to increasing the production and role of angiotensin (ANG II dependent pathway), it activates TGFβ, plasminogen activator inhibitor-1 (PAI-1), fibronectin, and collagen I independently from Ang II (ANG II independent pathway). Our group found that PRR contributed to liver fibrosis and HSC activation, and its down-regulation attenuated liver fibrosis through inactivation of the ERK/TGFβ1/Smad3 pathway. These results indicate that renin and prorenin can directly activate renin (pro) receptor-related intracellular signaling pathways, including ERK, TGFβ, cyclooxygenase2, fibronectin, collagen I, and PAI-1 independently of angiotensin II to induce fibrosis. Moreover, Ren et al. used N-acetylgalactosamine modified antisense oligonucleotides to suppress PRR expression in hepatocytes of high-fat diet-fed C57BL/6 mice, and found that PRR inhibition reduced acetyl-CoA carboxylase and pyruvate desorption hydrogenase protein expression. This change reprogrammed liver lipid metabolism, resulting in reduced lipid synthesis and increased fatty acid oxidation. As a result, liver PRR suppression attenuated diet-induced obesity and fatty liver. The proposed pathogenesis that is involved from steatosis to fibrosis in patients with NAFLD is shown in Figure 1.

PROGRESSION OF NASH TO HCC

NASH is now the most common risk factor for HCC in the United States. The potential pathways linking NASH to HCC include chronic inflammation of the liver, alternations in

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**Figure 1.** Progression of hepatic steatosis to inflammation and fibrosis in liver. Both metabolic and genetic factors contribute to the formation of hepatic steatosis. Fat accumulation in hepatocytes leads to organelles dysfunction and lipotoxicity. Then, oxidative stress species or signaling molecules are transmitted through extracellular vesicles or diffusion, activating other parenchymal and non-parenchymal cells, which subsequently causes inflammatory cascades, steatohepatitis, and liver fibrosis. On the other hand, gut-derived bacterial end-products, metabolites, gut hormones, adipose tissue-derived cytokines or adipokines, and renin-angiotensin system all contribute to the progression from steatosis to inflammation and fibrosis. SFA, saturated fatty acid; TG, triglyceride; ER, endoplasmic reticulum; HH-OPN, Hedgehog-osteopontin; KC, Kupffer cell; HSC, hepatic stellate cell.
immune response, lipid metabolism and gut microbiome, and genetic factors. Enhanced IL-6 and TNF production during NAFLD cause hepatic inflammation and activation of the oncogenic transcription factor STAT3. ER stress contributes to NASH-driven hepatic tumorigenesis via TNFR1. The hepatic oxidative DNA damage was increased in patients with NASH who developed HCC. The unconventional prefoldin RPBS interactor-induced DNA damage in hepatocytes triggered inflammation via T helper 17 lymphocytes and interleukin 17A, contributing to NASH and HCC development. Furthermore, NAFLD caused a selective loss of intrahepatic CD4(+) but not CD8(+) T lymphocytes, which led to accelerated hepatocarcinogenesis. Neutrophil infiltration was characterized in NASH-HCC and can exist in both tumor promoting and suppressing states. Fatty acid accumulation increased junctional protein associated with coronary artery disease, leading to the activation of Yes-associated protein 1 and tumor growth. Dysregulated mammalian target of rapamycin stimulated sphingolipid and glycerophospholipid synthesis, leading to steatosis and HCC. In NASH-driven HCC, metabolic reprogramming mediated by the downregulation of carnitine palmitoyltransferase 2 enables HCC cells to escape lipotoxicity and promotes hepatocarcinogenesis. MicroRNA-21 can promote hepatic lipid accumulation and cancer progression by interacting with the sHbp1-p53-Sreb1c pathway. The intestinal dysbiosis, gut permeability changes, and lipopolysaccharides translocation to the liver in NASH may increase secretion of the epieregulin growth factor, which triggers tumor hepatocyte proliferation. Moreover, carriage of the PNPLA3 rs738409 C>G polymorphism is associated with a greater risk of NASH-HCC.

**TREATMENT FOR NASH**

**Non-pharmacological therapy**

**Lifestyle modification**

Lifestyle changes by eating less and exercising more to achieve weight loss remain the cornerstone of clinical care. Hypocaloric diet with a reduction of body weight decreased total body fat, visceral fat, and intrahepatic lipid content. Some existing guidelines suggest restriction of energy by 1,200–1,500 kcal/day or a reduction of 500–1,000 kcal/day to achieve weight loss. Weight reduction is beneficial for both non-obese (3–10%) and obese patients (≥0%). Other dietary compositions that may be beneficial for NAFLD includes omega-3 PUFA and coffee. Omega-3 PUFA has been shown to increase insulin sensitivity and ameliorate steatohepatitis in experimental studies. One meta-analysis involving nine studies with 355 patients showed decreased liver fat in patients with PUFA treatment. Coffee is not only associated with a reduced risk of NAFLD but also decreased risk of liver fibrosis among patients with NAFLD. Regular exercise helps to enhance the effects of diet modifications. Physical activity with a target at least 150 min/week of moderate-intensity or 75–150 min/week of vigorous-intensity aerobic exercise is suggested. Both aerobic and resistance exercises reduce the hepatic fat content. In addition, the intensity of exercise may be more important than the duration or total volume. In conclusion, lifestyle interventions to promote weight loss, which include both diet and exercise, are proven therapeutic strategies to improve fatty liver disease.

**Surgery**

Bariatric surgery provides sustained and durable weight loss and improving obesity-related diseases. Currently, the most commonly performed bariatric procedures are laparoscopic sleeve gastrectomy (LSG) and Roux-en-Y gastric bypass. Two meta-analyses showed that bariatric surgery resulted in a biopsy-confirmed resolution of steatosis (56–66%), inflammation (45–50%), ballooning degeneration (49–76%), and fibrosis (25–40%), as well as reduction of NAFLD activity score (NAS). A higher rate of improvement in steatosis and hepatic fibrosis was observed in Asian countries compared to non-Asian countries. In addition, bariatric surgery was associated with decreased progression of NAFLD to cirrhosis and reduced risks of any cancer and obesity-related cancer in NAFLD patients with severe obesity, particularly in cirrhotic patients. However, new or worsening cases of NAFLD were found in 12% of patients after bariatric surgery. Bariatric surgery was associated with a significantly lower risk of incident major adverse liver outcomes (2.3% vs. 9.6% at 10 years) and major adverse cardiovascular events (8.5% vs. 15.7% at 10 years), as compared with non-surgical management.

**Endoscopic therapy**

Endoscopic bariatric therapies, including intragastric bal-
loons (IGB), endoscopic sleeve gastroplasty (ESG), duodenal mucosal resurfacing (DMR), and duodenal-Jejunal bypass lin-
er (DJBL), were recently introduced as less invasive modalities
to treat obesity and metabolic comorbidities. In a meta-
alysis, improvement in steatosis and NAS were seen in 79.2% and
83.5% of patients receiving IGB, respectively. Improvement of
defibrosis for 1.5 stage by MR elastography was seen in 50% of patients with NAFLD after IGB placement. ESG re-
duced the body weight by up to 15% and improved hepatic
steatosis and fibrosis at 2 years of follow-up in obese patients
with NAFLD. Studies for efficacy and safety of ESG
(NCT03426111; NCT04653311) and the comparison of ESG vs.
LSG (NCT04060368) in patients with NASH are ongoing. DMR
has been shown to reduce alanine aminotransferase, aspar-
ginate aminotransferase, and fibrosis-4 scores in patients with
diabetes mellitus. Recently, an observational study of 32
obese patients with diabetes mellitus who underwent DJBL
showed improved non-invasive markers of steatosis and
NASH, but not fibrosis. The role of DJBL on NAFLD needs to
be further evaluated.

Fecal microbiota transplantation

Some studies have suggested that fecal transplantation
helps ameliorate steatohepatitis. A randomized con-
trolled trial (RCT) using allogenic fecal microbiota transpla-
tation (FMT) from lean vegan donors for patients with NAFLD
through duodenal infusion found that there was no signific-
ant improvement in NAS, steatosis, and fibrosis scores. How-
ever, they observed a trend of improving necro-inflamma-
tory scores and beneficial changes in hepatic gene expression
and plasma metabolites involved in inflammation and lipid
metabolism following allogenic FMT. Another RCT using al-
logenic FMT via endoscopic duodenal infusion in patients
with NAFLD found that FMT did not improve insulin resis-
tance and hepatic steatosis but reduced small intestinal per-
meability at 6 months of follow-up.

Pharmacological therapy

The pharmacological agents predominantly target the fol-
lowing four mechanisms: 1) hepatic fat accumulation; 2) oxi-
dative stress, inflammation, and apoptosis; 3) gut-liver axis,
including bile acids, gut microbiomes, and metabolic endo-
toxemia; and 4) hepatic fibrosis. The agents targeting dif-
ferent pathways are described below, and those with promis-
ing results are summarized in Table 1.

Agents targeting hepatic fat accumulation

Pioglitazone, a PPAR γ agonist, improved hepatic steatosis,
inflammation, and hepatocellular ballooning. Similar ef-
cfects were found in Asian NASH patients. In the phase 3 RE-
SOLVE-IT trial, Elafibranor, a dual PPAR α/δ agonist, failed to
achieve NASH resolution. Pemafibrate, a selective PPAR α
modulator, did not decrease liver fat but caused a significant
reduction in fibrosis for 6.2% of magnetic resonance elastog-
raphy-based liver stiffness. Lanifibranor, a pan-PPAR ago-
nist, significantly decreased the steatosis-activity-fibrosis ac-
tivity score for at least 2 points in 55% of the patients at 24
weeks. GLP-1 agonists increase insulin secretion, inhibit glucagon
secretion, delay gastric emptying, and decrease appetite. NASH resolution was observed in 39% of patients who re-
ceived liraglutide for 48 weeks and in 59% of patients who received semaglutide for 72 weeks. However, fibrosis im-
provement was insignificant in both studies.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors increase
the urinary excretion of glucose. A meta-analysis of 10 RCTs
showed that SGLT2 inhibitors can reduce aminotransferases
and hepatic fat.

FGF19 and FGF21 are endocrines that regulate energy ho-
meostasis. Aldafermin, a FGF19 analogue, led to reductions
of liver fat content and a trend toward fibrosis improve-
ment. Pegbelfermin and efruxifermin are long-acting, re-
combinant analogues of human FGF21, and both have shown
effects of reducing liver fat.

Two phase IIa trials investigated the effects of acetyl-coen-
zyme A carboxylase (ACC) inhibitor monotherapy (PF-
05221304) and combination with a diacylglycerol O-acyl-
transferase 2 (DGAT2) inhibitor (PF-06865571). Both PF-
05221304 monotherapy and co-administration with PF-
06865571 reduced liver fat content.

Stearoyl-coenzyme A desaturase 1 (SCD-1) is a key enzyme
that catalyzes the biosynthesis of monounsaturated fatty acids.
A phase Iib trial (ARREST trial) showed that aramchol (a liver-targeted SCD-1 inhibitor) 600 mg did not cause a signifi-
cant reduction in liver fat content. Nevertheless, the ob-
served change in liver histology and biochemical improve-
ment suggests a potential role of aramchol in treating NASH
and fibrosis.

Thyroid hormone receptor-β (THR-β) is predominantly ex-
<table>
<thead>
<tr>
<th>Type of drug</th>
<th>Mechanism of action</th>
<th>Drug name</th>
<th>Study design</th>
<th>Study outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR agonist</td>
<td>PPAR-γ: ↑ insulin sensitivity modulates adipose tissue distribution, PPAR-α: ↑ fatty acid β-oxidation, PPAR-δ: anti-inflammatory</td>
<td>Pioglitazone</td>
<td>RCT; NASH, prediabetes/DM</td>
<td>↓ ALT/AST, ↓ Steatosis, ballooning necrosis, and inflammation, Fibrosis not improved</td>
<td>[188]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pioglitazone</td>
<td>vs. placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RCT; NASH</td>
<td>↓ ALT/GGT, Histology improvement, including liver injury and fibrosis</td>
<td>[189]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pioglitazone</td>
<td>vs. placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pemafibrate (SPPARα)</td>
<td></td>
<td>Phase II RCT; NAFLD and ↑ ALT</td>
<td>↓ ALT, ↓ Liver stiffness, No significant change of liver fat</td>
<td>[192]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pemafibrate</td>
<td>vs. placebo</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Lanifibranor (Pan-PPAR agonist)</td>
<td></td>
<td>Phase IIb RCT; NASH (SAF-A ≥3)</td>
<td>↓ SAF-A score ≥2 points in lanifibranor 1,200 mg group</td>
<td>[193]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lanifibranor</td>
<td>vs. placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 agonist</td>
<td>↑ Insulin secretion, ↓ Glucagon secretion, ↓ Gastric emptying, ↓ Appetite</td>
<td>Liraglutide</td>
<td>Phase II RCT; NASH, obesity</td>
<td>↑ Resolution of NASH without worsening of fibrosis, No difference in fibrosis improvement</td>
<td>[194]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liraglutide</td>
<td>vs. placebo</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Semaglutide</td>
<td>RCT, phase II; NASH (F1–F3 fibrosis), Semaglutide vs. placebo</td>
<td>↑ Resolution of NASH without worsening of fibrosis in semaglutide 0.4 mg group, No difference in fibrosis improvement</td>
<td>[195]</td>
</tr>
<tr>
<td>SGLT2 inhibitor</td>
<td>↑ Urinary excretion of glucose</td>
<td>SGLT2 inhibitors</td>
<td>Meta-analysis of 10 RCTs; NAFLD, DM SGLT2 inhibitor vs. other antidiabetic drugs</td>
<td>↓ ALT/AST, ↓ Liver fat content, visceral fat, and subcutaneous fat areas</td>
<td>[196]</td>
</tr>
<tr>
<td>FGF-19 analogue</td>
<td>↓ Bile acids synthesis, ↓ Hepatic gluconeogenesis, ↓ DNL, ↑ Fatty acid oxidation</td>
<td>Aldafermin</td>
<td>Phase II RCT; NASH (NAS ≥4, F2–F3 fibrosis, and liver fat content ≥8%) Aldafermin vs. placebo</td>
<td>↓ ALT/AST, ↓ Liver fat fraction on MRI-PDFF</td>
<td>[197]</td>
</tr>
<tr>
<td>FGF-21 analogue</td>
<td>↓ Hepatic gluconeogenesis, ↑ Insulin sensitivity, ↑ Energy expenditure, ↑ Mitochondria beta-oxidation in hepatocytes</td>
<td>Pegbelfermin</td>
<td>Phase IIa RCT; NASH (F1–F3 fibrosis, and liver fat content ≥10%), obesity Pegbelfermin vs. placebo</td>
<td>↓ Liver fat fraction on MRI-PDFF</td>
<td>[198]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pegbelfermin</td>
<td>vs. placebo</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Efruxifermin</td>
<td>Phase IIa RCT; NASH Efruxifermin vs. placebo</td>
<td></td>
<td>[199]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Efruxifermin</td>
<td>vs. placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of drug</td>
<td>Mechanism of action</td>
<td>Drug name</td>
<td>Study design</td>
<td>Study outcome</td>
<td>Reference</td>
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</tr>
<tr>
<td>Acetyl-CoA carboxylase inhibitor</td>
<td>↓ DNL, ↑ Fatty acid oxidation</td>
<td>PF-05221304</td>
<td>2 phase IIb RCTs; NAFLD/NASH PF-05221304 monotherapy vs. placebo</td>
<td>PF-05221304 monotherapy: ↓ Liver fat on MRI-PDFF, but ↑ TG</td>
<td>200</td>
</tr>
<tr>
<td>Diacylglycerol acyltransferase 2 inhibitor</td>
<td>↓ Synthesis of fatty acids into TGs</td>
<td>PF-06865571</td>
<td>PF-05221304 and PF-06865571 co-administration vs. placebo</td>
<td>Co-administration therapy: ↓ liver fat and mitigated ACC inhibitor-mediated effect on TG</td>
<td></td>
</tr>
<tr>
<td>Stearoyl-CoA desaturase 1 inhibitor</td>
<td>↓ DNL</td>
<td>Aramchol</td>
<td>Phase IIb RCT; NASH Aramchol vs. placebo</td>
<td>↓ Liver fat content in aramchol 600 mg group, but not significant</td>
<td>201</td>
</tr>
<tr>
<td>Selective thyroid hormone receptor-β agonist</td>
<td>↓ LDL, cholesterol, and TG, ↑ Fatty acid oxidation</td>
<td>Resmetirom</td>
<td>Phase II RCT; NASH (F1–F3 fibrosis, and liver fat content ≥10%) Resmetirom vs. placebo</td>
<td>↓ Liver fat on MRI-PDFF</td>
<td>202</td>
</tr>
<tr>
<td>Anti-oxidant</td>
<td>Anti-oxidative stress</td>
<td>Vitamin E</td>
<td>RCT; NASH, no DM Vitamin E vs. pioglitazone vs. placebo</td>
<td>↓ ALT/AST for both vitamin E and pioglitazone groups NASH improvement in vitamin E group, but not in pioglitazone group Fibrosis not improved in vitamin E and pioglitazone groups</td>
<td>204</td>
</tr>
<tr>
<td>Bile acid analogue</td>
<td>Anti-inflammation</td>
<td>Berberine ursodeoxycholate, Obeticholic acid (FXR agonist)</td>
<td>Phase II RCT; NAFLD, DM Berberine ursodeoxycholate vs. placebo Phase III RCT; NASH (NAS ≥4, F2–F3 fibrosis or F1 with ≥1 accompanying comorbidity) Obeticholic acid vs. placebo</td>
<td>↓ Liver fat content on MRI-PDFF ↓ Fibrosis No significant resolution of NASH</td>
<td>206</td>
</tr>
</tbody>
</table>

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PPAR, proliferator-activated receptor; SPPARMa, selective peroxisome proliferator-activated receptor α modulator; RCT, randomized controlled trial; ALT, alanine aminotransferase; SAF-A, steatosis-activity-fibrosis activity; GLP-1, glucagon-like peptide-1; SGLT2, sodium-glucose cotransporter 2; DM, diabetes mellitus; AST, aspartate aminotransferase; FGF, fibroblast growth factor; DNL, de novo lipogenesis; NAS, NAFLD activity score; MRI, magnetic resonance imaging; PDFF, proton density fat fraction; TG, triglyceride; ACC, acetyl-coenzyme A carboxylase; LDL, low density lipoprotein; FXR, farnesoid X receptor.
pressed in the hepatocytes. Resmetirom, a selective THR-β agonist, significantly reduced more than 30% of hepatic fat after 12 and 36 weeks of treatment in patients with NASH in phase II trial.²⁰²

**Agents targeting oxidative stress, inflammation, and apoptosis**

Vitamin E, an antioxidative agent, demonstrated benefits on hepatic decompensation and transplant-free survival in patient with NASH.²⁰³ The PIVENS study, which compared the effects of vitamin E, pioglitazone, and placebo in NASH patients without diabetes, showed that vitamin E (800 international units/day), but not pioglitazone, significantly improved NASH.²⁰⁴

Apoptosis signaling kinase 1 (ASK1) promotes apoptosis, inflammation, and fibrosis in the liver. However, selonsertib, an ASK1 inhibitor, failed to improve fibrosis in NASH patients with bridging fibrosis or compensated cirrhosis.²⁰⁵

Berberine ursodeoxycholate is an ionic salt of berberine and ursodeoxycholic acid. It reduced 4.8% of liver fat and improved glycemic control as well as liver enzymes in patients with NASH and diabetes.²⁰⁶

**Agents targeting gut-liver axis**

In a phase IIb study, obeticholic acid (OCA), a FXR agonist, improved liver histology in 21% of NASH patients.²⁰⁷ In patients with NASH and diabetes, OCA demonstrated the effects of increasing insulin sensitivity and reducing markers of liver inflammation as well as fibrosis.²⁰⁸ In the interim analysis of a phase III trial, both 10-mg and 25-mg doses of OCA improved fibrosis (18% and 23%, respectively), but the NASH resolution endpoint was not met.²⁰⁹ This study is ongoing to assess the clinical outcomes.

**Agents targeting liver fibrosis**

Caspase is a protease that is associated with apoptosis and inflammation in the liver. However, emricasan, a pan-caspase inhibitor, did not improve fibrosis or resolution of NASH.²¹⁰ Besides, for patients with NASH-related cirrhosis and severe portal hypertension, emricasan did not improve hepatic venous pressure gradient (HVPG) or liver-related outcomes.²¹¹

In a phase IIb CENTAUR trial, a 2-year study, cenocriprox, a dual C-C chemokine receptor types 2 and 5 antagonist, achieved ≥1-stage of fibrosis improvement without worsen-

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**Table 2. Ongoing phase III clinical trials of pharmacological agents in patients with NAFLD or NASH**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism</th>
<th>Patient</th>
<th>Outcome</th>
<th>Status</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanifibranor</td>
<td>Pan-PPAR agonist</td>
<td>NASH with stage 2–3 fibrosis without cirrhosis</td>
<td>NASH resolution; fibrosis improvement; liver-related events</td>
<td>Recruiting</td>
<td>NCT04894728</td>
</tr>
<tr>
<td>Semaglutide</td>
<td>GLP-1 agonist</td>
<td>NASH with stage 2–3 fibrosis without cirrhosis</td>
<td>NASH resolution; fibrosis improvement; liver-related events</td>
<td>Recruiting</td>
<td>NCT04822181</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>SGLT2 inhibitor</td>
<td>NASH and type 2 DM without cirrhosis</td>
<td>Histology improvement</td>
<td>Recruiting</td>
<td>NCT03723252</td>
</tr>
<tr>
<td>Resmetirom</td>
<td>Selective thyroid hormone receptor-β agonist</td>
<td>NASH without cirrhosis</td>
<td>NASH resolution; fibrosis improvement</td>
<td>Recruiting</td>
<td>NCT03900429</td>
</tr>
<tr>
<td>Aramchol</td>
<td>Stearoyl-CoA desaturase 1 inhibitor</td>
<td>NASH with stage 2–3 fibrosis without cirrhosis; type 2 DM or prediabetes</td>
<td>NASH resolution; fibrosis improvement</td>
<td>Recruiting</td>
<td>NCT04043221</td>
</tr>
<tr>
<td>Belapexin</td>
<td>Galectin-3 inhibitor</td>
<td>NASH cirrhosis without esophageal varices or gastric varices</td>
<td>Newly developed esophageal varices</td>
<td>Recruiting</td>
<td>NCT04368686</td>
</tr>
<tr>
<td>Oltipraz</td>
<td>Liver X receptor alpha-inhibitor</td>
<td>NAFLD without cirrhosis</td>
<td>Liver fat</td>
<td>Recruiting</td>
<td>NCT04142749</td>
</tr>
</tbody>
</table>
| NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PPAR, proliferator-activated receptor; GLP-1, glucagon-like peptide-1; SGLT2, sodium-glucose cotransporter 2; DM, diabetes mellitus.
ing of NASH after 1 year of treatment compared to placebo (20% vs. 10%). And a great proportion (60%) of the patients who achieved fibrosis response at the first year maintained fibrosis reduction at the second year. The long-term impact of cenicriviroc on fibrosis needs to be further investigated.

Belapectin, a galectin-3 inhibitor, did not significantly reduce HVPG or fibrosis in patients with NASH, cirrhosis, and portal hypertension; however, in a subgroup of patients without esophageal varices, belapectin reduced HVPG as well as the development of esophageal varices.

Information about the ongoing phase III clinical trials of promising drugs on phase II studies are listed in Table 2.

**Combination therapy**

NAFLD is a multifactorial disease, and combining therapies with different targets may have synergistic effects. Cilofexor (FXR agonist) plus firsocostat (ACC inhibitor) led to improvements in NASH activity compared to placebo, or single agent in patients with bridging fibrosis and cirrhosis. Semaglutide with firsocostat and/or cilofexor showed greater improvements in liver steatosis and liver biochemistry compared to semaglutide alone. Combining ACC inhibitors and DGAT2 inhibitors reduced liver fat content and mitigated the side effect of elevated serum TGs.

**PERSPECTIVES**

As understanding of mechanisms of NASH and its fibrosis increases, more therapies will be introduced and tested in clinical trials. The pathogenesis of NASH and fibrosis is complex; therefore, it would be difficult to treat the disease using just one therapy. Combination therapy is the focus in the future development of treatment. Furthermore, better care of extra-hepatic complications of NASH, novel biomarkers for diagnosis, risk stratification and treatment responses, and more clinical trials in Asian groups should also be well researched and developed.

**Authors’ contribution**

KC Lee and PS Wu drafted the manuscript and HC Lin revised the manuscript. All the authors read and approved the final version.

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**Conflicts of Interest**

The authors have no conflicts to disclose.

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96

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The emerging age-pattern changes of patients with hepatocellular carcinoma in Korea

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Keywords: Hepatocellular carcinoma; Aged; Epidemiology; Elderly

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide and the second leading cause of cancer-related death in Korea. Previous studies have reported that the incidence rate of HCC in Asia is more than 10-fold than that in Western countries. The highest age-adjusted incidence rates (>13.7–17.8 per 100,000) are recorded in East Asia (Korea, China, Mongolia, and Vietnam) and sub-Saharan Africa in 2020, which accounts for approximately 80% of liver cancer worldwide.

Hepatitis B virus (HBV) is still the predominant etiology of HCC in Korea, China, and Taiwan, accounting for approximately 60–70% of HCCs. The prevalence of chronic hepatitis B (CHB) ranged from 8% to 10% in the general population in the 1980s and early 1990s in Korea. However, owing to the universal vaccination program launched in 1983, the prevalence of CHB has significantly decreased. The Korea Advisory Committee on Immunization Practices also implemented the Hepatitis B Perinatal Transmission Prevention Program in July 2002. This program aimed to screen all pregnant women for CHB infection, provide prophylactic hepatitis B immunoglobulin to all infants born to hepatitis B surface antigen (HBsAg)-positive mothers, and vaccinate all infants against HBV. The HBsAg-positivity rates in the 10–18 years group markedly declined to 2.2% in 1998, 1.9% in 2001, 1.9% in 2007, and 0.3% in the Korea National Health and Nutrition Examination Survey 2016. Moreover, the use of potent nucleos(t)ide analogs significantly reduced the development of cirrhosis, leading to improved overall survival of patients with CHB. All of these effective strategies have changed the incidence patterns of HCC over time in Korea.

Chon et al. reported trends in HCC incidence in South Korea over 10 years (2008–2018) from the Korean National Health Insurance Service database (127,426 individuals) and predicted the incidence for the year 2028. From 2008 to 2018, the number and age-standardized incidence rates (ASRs; from 21.9 to 14.3 per 100,000 person-years) of HCC significantly decreased, except in older adults. Among individuals aged ≥80 years, the ASR significantly increased by 0.96% per year and the crude incidence rate of HCC also increased. From 2008 to 2018, the ASRs for individuals aged ≥80 years increased from 70.0 to 160.2 per 100,000 person-years.
Interestingly, Chon et al. also reported that the proportion of nonalcoholic fatty liver disease (NAFLD)-related HCC steadily increased from 2008 to 2018 (average annual percentage change, 2.7%; \( P<0.001 \)), with a rapid increase from 2011 to 2018 at an average of 5.6% per year. NAFLD-related hepatocarcinogenesis is indeed characterized by a long-lasting and insidious development.\(^6\)\(^7\) Patients with NAFLD-related HCC were generally older than those with virus-related HCC.\(^8\) HCC risk is higher in individuals with obesity\(^9\) and diabetes,\(^10\) as these conditions are two major risk factors for NAFLD. The pathogenesis of HCC in NAFLD is also independent of cirrhosis, and HCC in NAFLD might arise in the absence of fibrosis and histologically detectable inflammation. Obesity and excessive adipose tissue contribute to a chronic general low-grade inflammatory response, called lipotoxicity, and play an important role in hepatocarcinogenesis.\(^11\) The alteration of gut microbiota in patients with NAFLD also leads to hepatocarcinogenesis,\(^12\) which is affected by aging. Since Korea is a rapidly aging society, these factors might consistently increase the proportions of NAFLD-related HCC and older HCC patients.

The definition of “elderly” has become more difficult to agree. Generally, a chronological age of 65 years has been accepted as a threshold to define an “elderly” individual. In the scientific literature on HCC, the most commonly used threshold is 70 years.\(^13\) More recently, clinical studies adopting thresholds of 75 or 80 years have been published.\(^14\) The increasing age of patients with HCC brings some drawbacks to choosing treatment modality due to the occurrence of comorbidities, which can be associated with reduced tolerability and an increased risk of serious adverse events. The Eastern Cooperative Oncology Group performance status, which quantifies constitutional syndrome due to tumor burden, is one of the key factors that determine disease stage, consequently influencing the choice of treatment modality. Another problem frequently encountered in elderly patients with HCC is that they are relatively reluctant to undergo surgical resection or systemic therapies, erroneously considered too risky for older patients. Clinical trials specifically designed to compare HCC outcomes in older patients aged \(>75\) years are lacking. IMbrave150 trial also included subjects with aged \(\leq 71\) years.\(^15\) The available data on HCC outcomes in older patients with HCC are mainly from retrospective observational studies. Long-term survival in elderly patients with HCC is mainly dependent on their expected shorter life span than younger patients and the occurrence of comorbidities. Kim et al.\(^16\) reported a retrospective Korean HCC cohort study showing that non-liver-related mortality was significantly higher in older patients (\(\geq 70\) years) than in younger patients, although the overall survival was similar to that found in patients aged \(<70\) years. Therefore, the allocation of treatment modalities should be determined according to HCC stage, liver function, and performance status,\(^17\) rather than chronological age. Chronic age \(\geq 75–80\) years is not an absolute contraindication for surgical resection or systemic therapy. Older patients with resectable tumors and well-preserved liver function may benefit from surgical resection. Systemic therapy may also be a viable option for treating advanced HCC in older patients. Clinicians should carefully evaluate concomitant comorbidities, particularly cardiovascular diseases. Previous studies reported that tyrosine kinase inhibitors, including sorafenib and lenvatinib, were also effective and tolerable for older patients with HCC aged \(>70–75\) years with more vigilant monitoring.\(^18\) A recent multicenter analysis from Japan (\(n=317\)) also reported the safety and efficacy of atezolizumab plus bevacizumab in older patients with HCC aged \(\geq 75\) years. In a subgroup analysis of older patients aged 75–79, 80–84, and \(\geq 85\) years, w no significant differences were found in cumulative overall or progression-free survival and treatment-related adverse events among these age groups.\(^19\) However, a recent multicenter retrospective observational study from Japan showed poorer tolerability to lenvatinib in older patients aged \(\geq 80\) years than in patients aged \(<80\) years. Therefore, meticulous management\(^20\) of adverse events is crucial for the adherence and maintenance of systemic therapies in older patients with HCC.

Changes in the epidemiology of chronic liver disease have led to changes in the age at HCC diagnosis in Korea. The proportion of older patients with HCC is gradually increasing. Additionally, Chon et al.\(^6\) reported that by 2028, the number of patients with HCC aged \(\geq 80\) years will be greater than the number of HCC patients in 2008. It is not appropriate to re-

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**Abbreviations:**

ASR, age-standardized incidence rate; CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease

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strict the upper age limit for the HCC surveillance. Allocation of treatment modality should be determined according to HCC stage, liver function, and performance status, rather than chronological age.

Authors’ contribution
Study conceptualization: YC and JWP; Drafting of the manuscript: YC; Critical revision of the manuscript: BHK, YC, and JWP.

Conflicts of Interest
The authors have no conflicts to disclose.

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The rise of non-invasive tools in the diagnosis of portal hypertension: Validation of the Baveno VII consensus

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Keywords: Hypertension, portal; Liver cirrhosis; Baveno consensus

Since its inception in 1986 by professor Robert de Franchis, the Baveno consensus is published every 5 years and has become the world’s most-cited diagnostic guideline for portal hypertension.1 Signiﬁcant changes were made in the Baveno VII consensus released in 2021 compared to the 2015 Baveno VI consensus.2,3 Table 1 is the comparison of the Baveno VI and VII criteria showing the unique features of the Baveno VII. Conditions like compensated advanced chronic liver disease (cACLD) and clinically signiﬁcant portal hypertension (CSPH) are emphasized in both Baveno VI and VII. However, in Baveno VII, i) hepatic venous pressure gradient (HVPG) measurement and clinical importance were further underscored, ii) usefulness of transient elastography (TE) as a non-invasive test was highlighted, and iii) a cut-off for spleen stiffness to estimate CSPH was presented.2

The Baveno consensus is based on the results of recent research such as ANTICIPATE study and PREDESCI study.4,5 However, the criteria of cACLD or CSPH based on a non-invasive test need to be validated in a large cohort to verify the clinical utility of the cut-offs for real-world applications.6,7 Recently, a study was conducted to validate the recompensation criteria of the Baveno VII,8 but few studies thus far have validated the CSPH criteria based on a non-invasive assessment. At an opportune time, Wong et al.9 conducted a large-scale multinational study to validate the CSPH criteria based on TE and platelet count in cACLD patients (TE value ≥10 kPa). In this study, CSPH was classiﬁed into four groups. First two groups were: i) deﬁnite CSPH, TE >25 kPa; ii) excluded CSPH, TE <15 kPa and platelet count ≥150×10⁹/L. If patients do not meet either of the first two criteria, they were classiﬁed as grey zone while patients in the grey zone were categorized into two groups; iii) high probability of CSPH, TE value between 20–25 kPa and platelet count <150×10⁹/L, or TE value between 15–20 kPa and platelet count <110×10⁹/L; and iv) low probability of CSPH (remainder of the patients within the grey zone who do not meet the high probability of CSPH condition).

Wong et al.9 showed that the deﬁnite CSPH (TE >25 kPa) criterion can effectively predict liver decompensation and
liver-related events. In particular, the fact that any patient with the excluded CSPH condition did not experience decompensation augments the reliability of the exclusion criteria. It is worth noting that the condition of high probability of CSPH was associated with multiple patterns of CSPH-related symptoms depending on etiology. In the grey zone, patients with viral etiology rarely experienced hepatic decompensation, whereas patients with non-viral etiology had a relatively increased risk of hepatic decompensation. This interpretation is based on the fact that all of the viral etiology patients enrolled in the study of Wong et al. were in the state of viral suppression by antiviral treatment. On the other hand, the increased risk of non-viral etiology despite the exclusion of all patients with significant alcohol consumption in the study suggests that the risk of hepatic decompensation in the grey zone may be markedly higher in real clinical practice dealing with cases of active alcoholic cirrhosis. Regarding the grey zone, more empirical research is needed to examine the feasibility of a fine-tuned scheme of CSPH cut-off for each etiology. For a more accurate prognosis of the CSPH among the patients within the grey zone, the spleen stiffness (SS) can be jointly considered in line with the proposition of the recent studies. The use of SS was recommended for the first time in the Baveno VII, and SS can be used in viral hepatitis cACLD patients to rule out (SS <21 kPa) and rule in (SS >50 kPa) CSPH. Therefore, if SS values are used in combination with TE and platelet counts, the gray zone condition can be further subdivided. In particular, the results of SS would be promising because the current criteria for CSPH (TE >5 kPa) cannot be used for obese (body mass index >30 kg/m^2) patients with NASH-associated cirrhosis. The costly process of measuring SS in a large number of cACLD patients remains a challenge, though.

The criteria for recommending the non-selective beta-blockers (NSBBs) in the Baveno VII were not clearly defined. In this study, decision curve analysis demonstrated that an overall net benefit of using NSBB in cACLD patients is largest when treating only CSPH (HVPG ≥10 mmHg) rather than treating all varix or the probable CSPH group. Similarly, NSBB was used only in the compensated cirrhosis with CSPH (HVPG ≥10 mmHg) patients in the PREDESCI study. To identify the patient group in which the NSBB is clinically helpful, more studies are needed to further refine the cut-offs.

The most notable limitation of this study is that four cohorts with high heterogeneity were pooled. Specifically, the distribution of etiology markedly different across the four countries (i.e., Italy, hepatitis C only; Singapore, hepatitis C dominant; India and China, hepatitis B dominant) is likely to be a confounding factor. Also, patients with active viral hepatitis and active alcohol drinking were excluded and few

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**Table 1. Comparison of the Baveno VI and VII criteria for cACLD and CSPH**

<table>
<thead>
<tr>
<th></th>
<th>Baveno VI</th>
<th>Baveno VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclude cACLD</td>
<td>TE value &lt;10 kPa</td>
<td>TE value &lt;10 kPa in the absence of other known clinical/imaging signs</td>
</tr>
<tr>
<td>Suggestive of cACLD (grey area)</td>
<td>TE value 10–15 kPa</td>
<td>TE value 10–15 kPa</td>
</tr>
<tr>
<td>Assume cACLD</td>
<td>TE value &gt;15 kPa</td>
<td>TE value &gt;15 kPa</td>
</tr>
<tr>
<td>Exclude CSPH in patients with cACLD</td>
<td>Not stated</td>
<td>TE value ≤15 kPa and platelet count ≥150×10^9/L</td>
</tr>
<tr>
<td>Assume CSPH in patients with cACLD</td>
<td>TE value ≥20–25 kPa, alone or combined to platelets and spleen size</td>
<td>TE value ≥25 kPa, alone (not applicable to obese [BMI &gt;30 kg/m^2] patients with NASH cirrhosis)</td>
</tr>
<tr>
<td>Need for screening endoscopy</td>
<td>TE value ≥20 kPa or platelet count ≤150×10^9/L</td>
<td>TE value ≥20 kPa or platelet count ≤ 150×10^9/L</td>
</tr>
<tr>
<td>Spleen stiffness</td>
<td>Not stated</td>
<td>Can be used in cACLD due to viral hepatitis to rule out (SSM &lt;21 kPa) and rule in (SSM &gt;50 kPa) CSPH</td>
</tr>
</tbody>
</table>

**Abbreviations:**
cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; NASH, nonalcoholic steatohepatitis; NSBB, non-selective beta-blocker; SS, spleen stiffness; TE, transient elastography
obese NASH patients were included in the study, potentially limiting the generalizability of the cut-offs for CSPH in a broad range of patients.

The Baveno VII consensus and this study demonstrate the expanding application of TE to the prediction of cACLD, CSPH, and determination of NSBB appropriateness. In medical fields, there are trends of gradually decreasing use of invasive methods such as HVPG and endoscopy, and increasing use of non-invasive approaches. In the future, it is expected that various cut-offs for portal hypertension would be developed in consideration of shear wave elastography and spleen stiffness measurement as well as TE. Although endoscopy is a useful tool for variceal surveillance, it is recommended to patients only when it is inevitable. Compared to other Western countries, endoscopy is easier to access and relatively inexpensive in South Korea due to its unique health care system. That being said, some potential adverse effects of endoscopy such as emission of greenhouse gases can be mitigated by the use of endoscopy for sensibly targeted patients only.

Authors’ contribution
Jeong-Ju Yoo contributed to write the manuscript. Sang Gyune Kim contributed to study concept and writing.

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Conflicts of Interest
The authors have no conflicts to disclose.

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Editorial

Non-invasive tests-based risk stratification: Baveno VII and beyond

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\textbf{Keywords:} Hypertension; Portal elasticity imaging techniques; Liver cirrhosis

Portal hypertension is the main driver of complications in compensated advanced chronic liver disease (cACLD). Particularly, the presence of clinically significant portal hypertension (CSPH) identifies those at risk for hepatic decompensation.\textsuperscript{1}

The recommendation of the recent Baveno VII consensus to treat CSPH upon diagnosis (i.e., prevention of hepatic decompensation), instead of waiting for (high-risk) varices to develop to initiate primary bleeding prophylaxis, marked a paradigm shift in clinical hepatology.\textsuperscript{2} Specifically, endoscopies to screen for high-risk varices or performance of endoscopic band ligation have been a cornerstone in the management of cACLD patients (i.e., primary prophylaxis of variceal bleeding) until recently.\textsuperscript{3,4} Following Baveno VII,\textsuperscript{2} the presence of CSPH should be investigated by non-invasive tests (NIT)\textsuperscript{5} or, where available, hepatic venous pressure gradient-measurement\textsuperscript{6} upon the diagnosis of cACLD, to facilitate timely treatment initiation with non-selective betablockers (NSBB; preferably carvedilol) for preventing first hepatic decompensation\textsuperscript{1,7,8} - most commonly, the occurrence of ascites.\textsuperscript{2}

In a recent issue of Clinical and Molecular Hepatology, Wong and colleagues\textsuperscript{9} set out to validate the performance of NIT to exclude CSPH (liver stiffness measurement [LSM] <15 kPa and platelet count [PLT] ≥150×10\textsuperscript{9}/L), rule-in CSPH (LSM ≥25 kPa), and to identify those at high probability for CSPH (LSM 20–25 kPa and PLT <150×10\textsuperscript{9}/L, or LSM 15–20 kPa and PLT <110×10\textsuperscript{9}/L) for the prediction of first hepatic decompensation in a multicentre cohort, including 1,159 ‘cACLD’ patients from Italy, India, China, and Singapore. Notably, this multi-ethnic cohort included predominantly cured hepatitis C virus (HCV) (56%) or suppressed hepatitis B virus (HBV) (21%) patients; the median LSM was approximately 24 kPa at study inclusion, and the patients were followed for 40 months.

The authors report on several important aspects: patients in whom CSPH could be ruled-in (LSM ≥25 kPa; 37% of the
study population) had a substantially increased risk of first hepatic decompensation within the follow-up period across all aetiologies (14% at 3 years). At the same time, patients in whom CSPH could be excluded (LSM <15 kPa and PLT ≥150x10^9/L) did not develop any hepatic decompensation during follow-up, yet a considerable proportion developed hepatocellular carcinoma. However, patients within the diagnostic/prognostic ‘grey-zone’ (i.e., not falling into one of these categories; 51% in this study) still had a relevant risk to develop first hepatic decompensation, especially in non-viral aetiologies. Also, using the selected criteria for a ‘high probability of CSPH’ within this grey-zone did not introduce any granularity, and was, therefore, insufficient for prognostication.

These data clearly support the utility of NIT-based criteria to rule-in or exclude CSPH and stratify the risk of its clinical sequelae in everyday practice, as they identify subsets of patients with profoundly different risks of first hepatic decompensation. However, a large proportion of patients (>50%) fall into neither category, leaving them unclassified. Here, recent approaches have introduced spleen stiffness measurement (SSM)\textsuperscript{10} or the ratio of von Willebrand factor (VWF) and PLT (VITRO score)\textsuperscript{11} to close this diagnostic gap (Fig. 1; 1st scenario).\textsuperscript{12,13} Specifically, combining Baveno VII criteria (as outlined above) with SSM ≤40 kPa/>40 kPa,\textsuperscript{12} or sequentially applying Baveno VII criteria and a VITRO-score ≤1.5 or ≥2.5 reallocated up to 75% of previously unclassified patients into the ruled-out/in category while maintaining a high diagnostic accuracy, thereby reducing the grey-zone for CSPH to only 10–15% of all cACLD patients.\textsuperscript{12,13} Most importantly, both SSM- and VITRO-based approached were also able to discriminate between patients at risk vs. those not at risk for first hepatic decompensation.\textsuperscript{12,13} The sequential application might especially be important to identify ‘at-risk’ patients with CSPH who are otherwise missed by LSM alone. While the ≥25 kPa cut-off is generally endorsed across all etiologies, the optimal cut-off for CSPH might vary across etiologies,\textsuperscript{14-16} prompting other NIT, such as SSM/VWF/VITRO, that

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**Figure 1.** Three different approaches (i.e., scenarios) to improve risk stratification in the diagnostic grey-zone of clinically significant portal hypertension (CSPH). LSM, liver stiffness measurement; PLT, platelet count; NIT, non-invasive tests; VITRO, ratio of von Willebrand factor and platelet count; SSM, spleen stiffness measurement. *Modified from Dajti et al. (2022) requesting 2 of 3 criteria to exclude CSPH (LSM <15 kPa and PLT ≥150x10^9/L or SSM ≤40 kPa) and 2 of 3 to rule-in CSPH (LSM ≥25 kPa and PLT <150x10^9/L or SSM >40 kPa).

**Abbreviations:**
ALD, alcoholic liver disease; cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; HBV, hepatitis B virus; HCV, hepatitis C virus; LSM, liver stiffness measurement; NIT, non-invasive tests; NNT, number-needed-to-treat; NSBB, non-selective beta blocker; PLT, platelet count; SSM, spleen stiffness measurement; VITRO, ratio of von Willebrand factor and platelet count; VWF, von Willebrand factor

better reflect the dynamic component of portal hypertension.\textsuperscript{17}

In general, the inclusion of ‘cACLD’ patients in whom the primary aetiological factor has been removed (i.e., HCV, 56%; alcoholic liver disease [ALD], 9%; patients with reported ongoing abuse were excluded) or suppressed (HBV, 21%) as done by Wong and colleagues\textsuperscript{2} might indeed be reflective of today’s ‘real-world’ practice, yet it introduces heterogeneity in the underlying risk for first hepatic decompensation. Here, numerous studies have shown that the risk of first hepatic decompensation is considerably lower after HCV-cure,\textsuperscript{18} and that specific risk stratification algorithms for CSPH\textsuperscript{19} but also first hepatic decompensation (e.g., by combining LSM and VITRO) are required\textsuperscript{20,21} while the overall accuracy of NIT for CSPH is generally comparable.\textsuperscript{22} To account for these peculiarities, the term ‘cACLD’ as currently defined by Baveno VII even explicitly excludes patients after removal of the primary aetiological factor (‘the term cACLD had been proposed to reflect the continuum of severe fibrosis and cirrhosis in patients with ongoing chronic liver disease’)--which was not accounted for in this study.

Most interestingly, non-viral aetiology (which basically reflects the absence of ‘removal of the primary aetiological factor’) had a stronger impact on first hepatic decompensation risk as compared to having a high probability of CSPH (i.e., the disease stage) at baseline (subdistribution hazard ratio, 3.25 vs. 2.48). This underscores the profound change in underlying risk achieved by aetiological cure, as it already provides a glimpse into the likely future of the patient (i.e., progressive vs. regressive disease). At the same time, this strongly argues for the incorporation of the concept of ‘aetiology’/‘removal of the primary aetiological factor’ into risk stratification models (Fig. 1; 2nd scenario). Alternatively, NITs offer the unique opportunity to longitudinally monitor disease dynamics (progression vs. regression), and therefore, repeated re-staging (Fig. 1; 3rd scenario). Here, it remains to be shown whether the underlying risk may be even better captured by repeating NITs (i.e., NIT trajectories) and whether the consideration of these trajectories outperforms concepts of ‘aetiology’/‘removal of the primary aetiological factor’ alone.

Importantly, patients with NSBB treatment at baseline were excluded from this study. However, this may underestimate the risk of first hepatic decompensation among CSPH ruled-in patients encountered (but not scoped) in recent clinical practice, as patients with high-risk varices (and thus, most severe portal hypertension/highest decompensation risk) were underrepresented by design. Also, patients were not censored at the time of the initiation of prophylactic treatment (in particular, NSBB therapy), which may also have decreased the first hepatic decompensation risk.

Compared to the “PREDESCI” study, and as discussed by the authors, the risk of first hepatic decompensation was considerably lower in the study by Wong and colleagues\textsuperscript{9} (24% in the placebo group of the “PREDESCI” study vs. 13.3% of CSPH ruled-in patients from this study).\textsuperscript{23} While this can be explained by differences in the underlying patient population (patients with CSPH vs. CSPH ruled-in by NIT; only active HCV infection in the PREDESCI trial vs. only cured HCV patients in the present study), it also influences the number-needed-to-treat (NNT) for NSBB, which might be even lower than in the study by Wong and colleagues\textsuperscript{9} (proposed NNT of 27–50). This calls for a ‘non-invasive’ PREDESCI trial to re-ensure our current clinical practice using contemporary patients.

Finally, it remains unclear how regional differences in healthcare might have confounded the results of our study, as aetiologies showed profound geographical clustering: 59%/27% of all HCV patients were treated in Italy/Singapore; 57% of all HBV patients were treated in China, 86% of all ALD patients were treated in India; and 68% of all NASH-patients were treated in India. Since including patients from around the globe does not guarantee the generalizability of the findings to a specific region, evaluating geographical regions/aetiologies independently might be another important task for future studies.

All things considered, the study by Wong et al.\textsuperscript{9} is an important proof-of-concept, indicating that non-invasive risk stratification for CSPH is valid across different aetiologies, ethnicities, and countries, as it identifies patients at risk for first hepatic decompensation who may benefit from NSBB treatment (CSPH ruled-in), and those at negligible risk of hepatic decompensation (CSPH excluded). More granular information is required to optimize risk stratification/treatment allocation in the broad diagnostic grey-zone of the Baveno VII recommendations; however, specifically designed NIT-based approaches (SSM\textsuperscript{12} and VITRO\textsuperscript{13}) have already been added to our armamentarium. Finally, a randomized controlled trial would be desirable to provide a definite proof for the Baveno VII approach to use NSBB treatment to prevent first hepatic
decompensation in patients in whom CSPH has been ruled-in non-invasively.

Authors’ contribution
Drafting of the manuscript (G.S., M.J., M.M.), critical revision of the manuscript for important intellectual content (G.S., M.J., M.M.).

Conflicts of Interest
The authors have no conflicts to disclose.

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For over six decades, hepatic venous pressure gradient (HVPG) has been the established method to assess accurately portal pressure in patients with cirrhosis and the best predictor of decompensation in compensated patients. Given its invasive nature and reduced accessibility in most centers, HVPG is limited in its widespread applicability. The appearance of noninvasive tests (NITs), most notably, transient elastography, catapulted these methods from diagnostic and staging tools to prognostic markers for the evaluation of portal hypertension. In the previous Baveno VI, NIT criteria were defined to stratify patients with high-risk varices, sparing endoscopies. The most recent Baveno VII took the role of NITs in patients with clinically significant portal hypertension (CSPH) one step further by defining cut-offs for the presence of CSPH and prognosis, risk stratification and indication for start of beta-blocker therapy.

In a recent issue of Clinical and Molecular Hepatology, Wong et al. reported, in a retrospective cohort study, that one-third of compensated advanced chronic liver disease (cACLD) patients fulfilled the non-invasive criteria of CSPH defined in the Baveno VII Consensus Workshop. These criteria had yet to be evaluated to predict the risk of decompensation, making this a very timely study. The authors found that, although the noninvasive assessment of CSPH predicts first liver decompensation (variceal bleeding, clinically overt ascites and overt hepatic encephalopathy), and the need for non-selective beta-blocker (NSBB) in cACLD patients, for patients in the category “probable CSPH” these criteria were suboptimal to predict decompensation in cACLD patients. Over three-fourths of the patients included in this multicentre study had treated hepatitis B or C-associated cACLD. In this study, the Baveno VII criteria to define CSPH (liver stiffness measurement [LSM] ≥25 kPa) and exclude it (LSM <15 kPa and platelet count [PLT] ≥150×10⁹/L) were used. Grey zones were classified into two groups: (high – LSM between 20–25 kPa and PLT <150×10⁹/L, or LSM between 15–20 kPa and PLT <110×10⁹/L, or low – defined as the remaining patients within the grey zone). Within a median follow-up of 40 months (30–52), among the 1,159 cACLD patients, 7.2% developed a first decompensation (ascites, variceal bleeding or hepatic encephalopathy), 5.8% hepatocellular carcinoma and 4.4%
died. Decision curve analysis to assess various screening strategies to stratify patients for NSBB to prevent decompensation showed treating definite CSPH (LSM ≥25 kPa) as a superior strategy was to “treating probable CSPH” and “treating any varices” to initiate NSBB. The number needed to treat was 27 and 50, at treatment thresholds of 5% and 10%, respectively.8

The Baveno VII consensus cut-offs were based on previous studies, such as the ANTICIPATE study which showed that using NITs, namely LSM combined with PLT showed an excellent discriminative value (AUC, 0.85) in patients with Child-Pugh A compensated cirrhosis. A subsequent study including more patients with non-alcoholic steatohepatitis (NASH) demonstrated that a LSM ≥25 kPa is sufficient to rule in CSPH in most aetiologies, including nonobese patients with NASH, but not in obese patients with NASH.10 The “rule of five” for LSM, at 5–10–15–20–25 kPa, is a tool to stratify the risk of liver-related events, and LSM alone or in combination with PLT, was presented at the recent Baveno VII meeting, and can be used to rule-in and rule-out cACLD and CSPH, as well as to rule-out high-risk varices.6 During the preparation for the Baveno VII consensus, an individual patient data meta-analysis was performed in patients with cACLD after hepatitis C virus (HCV) eradication, with paired HVPG and LSM, and recently published.11 Regarding the association between NITs and HVPG, a stronger correlation between LSM and HVPG was observed after HCV cure than in patients with active HCV infection, and similar for PLT and HVPG.11 Furthermore, LSM/PLT ratio for CSPH was comparable or tended to be even better after HCV cure compared to pre-treatment (AUC 0.753 vs. 0.800 for PLT, 0.831 vs. 0.837 for LSM, and 0.871 vs. 0.884 for both).11 The authors applied these criteria for LSM and PLT to predict decompensation in a validation cohort of cACLD patients. The 3-year decompensation risk was 0% in patients who met the LSM <12 kPa and PLT >150 g/L criteria. In patients with LSM ≥25 kPa, the 3-year decompensation risk was 9.6%. Among the 40.7% between these cut-offs, only 1.3% developed a decompensation.11

There are several limitations of the study by Wong et al.8 Among these, are the variability in patients’ characteristics and clinical practices across the different institutions, the retrospective nature of the study introduces particularly information bias, not accounting for missing information in patients’ records. As the authors mentioned over 75% of the patients included had treated viral cACLD, the applicability of these criteria for patients with other aetiologies remains unclear. In this study, the non-viral aetiology, such as alcohol- or metabolic-related, was identified as one driving factor of liver decompensation, which is unsurprising. The study leaves questions regarding the role “active versus treated disease”, regarding the applicability of the Baveno VII criteria.

The study by Wong et al.9 has highlighted the need to better stratify the 40–50% of cACLD patients that belong to the “grey zone” of LSM 15–25 kPa for CSPH according to the Baveno VII criteria. In fact, pilot data from a recent retrospective study, using an algorithm combining spleen stiffness measurement (cut-off >/<40 kPa) with the latest rule in and rule out CSPH criteria (LSM ≤15 kPa + PLT ≥150 g/L to rule out CSPH and LSM >25 kPa to rule in CSPH), reduced the grey zone from 40–60% to 7–15%. All first decompensation events occurred in the “rule-in” zone of the model including spleen stiffness measurement.12 Besides combining with other tools to refine the patients at risk, these criteria require validation in patients with nonviral causes for cACLD, particularly in obese NASH patients, where LSM is not as accurate.10 Additionally, the role cofactors, such as obesity and diabetes, play in disease progression and LSMS values is a matter of further investigation. Apart from expanding NITs in different aetiologies,13 there is a need for further validate NIT other than transient elastography in cACLD. Another point to explore is the validation of the use of deltas of LSM alone or in combination with other NITs, such as Fibrosis-4 index, and which reduction in stiffness, constitutes an improvement with clinical significance. Recent data has shown that a percentage drop in stiffness, 10% or 20%, can help predict liver-related events.14,15 Until date, very few studies have analysed the use of NITs alone or in combination with other markers, to evaluate response to beta-blocker therapy.16,17 Despite the grey areas that remain, the recent Baveno VII criteria have sent the ball rolling to expand the role of NITs in the clinical management of patients, solidifying their crucial role in patients with cACLD.

**Abbreviations:**
cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; HCV, hepatitis C virus; HVPG, hepatic venous pressure gradient; LSM, liver stiffness measurement; NASH, non-alcoholic steatohepatitis; NIT, noninvasive test; NSBB, non-selective beta-blocker; PLT, platelet count

Conflicts of Interest
The author has no conflicts to disclose.

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Editorial

Moving toward hepatitis B virus functional cure - the impact of on-treatment kinetics of serum viral markers

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Keywords: Hepatitis B virus; Hepatitis B; Antiviral agents

BACKGROUND

Functional cure of chronic hepatitis B virus (HBV) infection, which is currently set as the treatment goal of new HBV therapies, is serologically defined as the clearance of hepatitis B surface antigen (HBsAg), with or without anti-HBs seroconversion, and undetectable serum HBV DNA.¹ A handful of studies have shown that patients with chronic hepatitis B (CHB) who achieve functional cure generally have a favorable clinical course – namely much reduced risk of hepatic events and hepatocellular carcinoma (HCC).² Nonetheless, there is still a low yet definite risk of HCC occurrence, especially in male patients who achieve functional cure after 50 years of age.¹ While the current antiviral treatment with oral nucleos (t)ide analogues (NAs) are potent and safe, they generally lead to very low rates of functional cure; hence, novel HBV therapeutic regimens are eagerly wanted for improving the functional cure rate.³⁴ Before achieving functional cure, the holy grail of treatment goals, favourable HBsAg response (FHR) is a reasonable intermediate step towards HBV cure. FHR was defined as HBsAg seroclearance or HBsAg ≤100 IU/mL at the end of follow-up (EOFU). Such a low HBsAg cutoff is often adopted for stopping NA therapy in hepatitis B e antigen (HBeAg)-negative patients, as their relapse rate would be low.⁶ End-of-treatment HBsAg <100 IU/mL is also one of the few virologic predictors of functional cure.³ Several studies have investigated the functional cure rate after stopping NA in HBeAg-negative patients, with variable rates of success ranging from 2.7–16.7%/year in Caucasian patients and 0–3.8%/year among Asian patients; the most consistent predictor of functional cure is a low HBsAg level at the time of NA withdrawal.¹

KEY FINDINGS

Overview of study methodology

Mak and colleagues⁷ examined the serum hepatitis B core-
related antigen (HBcrAg) and HBV pre-genomic RNA (pgRNA) in the first 48 weeks of NA treatment in 64 CHB patients. Analyses were performed separately in 28 HBeAg-positive and 36 HBeAg-negative patients. These patients had participated in previous phase III trials, during which they received lamivudine, adefovir dipivoxil, telbivudine, clevudine, or entecavir with serum samples collected at weeks 0, 4, 12, 24, 36, and 48 of treatment and paired liver biopsies at weeks 0 and 48. HBcrAg was measured with a lower limit of detection (LLOD) of 2 log U/mL and a reliable quantification at >3 log U/mL. HBV RNA was measured using an investigational assay with a LLOD of 1 log copies/mL. Patients with undetectable serum HBV pgRNA or HBcrAg at week 0 were excluded from the analysis of early on-treatment changes of viral markers. Due to censoring and the difference in follow-up durations between patients with and without FHR at the EOFU, time-dependent area under receiver operating curve was utilized to assess the discriminatory ability of HBcrAg and HBV pgRNA.

Clinical meaning of HBV pgRNA decline

At a median follow-up duration of 17 years, 22/64 patients achieved FHR, including eight cases of HBsAg seroclearance. At the start of NA therapy, HBeAg-positive patients had higher HBV pgRNA compared to HBeAg-negative patients (4.9 vs. 3.3 log copies/mL). The median HBV pgRNA reduced to the LLOD at week 48 and week 12 for HBeAg-positive and HBeAg-negative patients, respectively. The difference in median reduction of HBV pgRNA between FHR and non-FHR patients was prominent among HBeAg-positive patients, with over 1 log difference sustained between week 4 and week 48. Patients with FHR had a higher HBV pgRNA in the first 12 weeks than those without FHR, while the level was comparable after week 24. The decline of HBV pgRNA at weeks 4 and 48 were the most discriminative of FHR. In contrast, among HBeAg-negative patients, the HBV pgRNA level and its reduction did not significantly differ throughout the first 48 weeks between patients with and without FHR.

Serum HBV RNA is a novel biomarker that can measure the circulating HBV pgRNA present in virus-like particles.8,9 Serum HBV RNA is a mixture of intact, pre-genomic and subgenomic, spliced, truncated, and polyA-free species,10 which reflects the transcriptional activity of intrahepatic covalently closed circular DNA (cccDNA).11 HBV RNA level at NA treatment cessation also correlates with post-treatment viral relapse.11 Notably, while there was a significant reduction of HBV pgRNA, no significant differences in the reduction of intrahepatic total HBV DNA and cccDNA level were observed at week 48 between HBeAg-positive patients with and without FHR.7 One may speculate that cccDNA transcriptional activity is more predictive of FHR than its absolute amount. Nevertheless, the difference in the role of pgRNA in HBeAg-positive and HBeAg-negative patients remain unclear. Also, assays for serum HBV RNA should be standardized and validated to better define its clinical utility.11

Clinical meaning of HBcrAg decline

Mak and colleagues7 also demonstrated the importance of early decline in serum HBcrAg at week 4 during antiviral treatment, which was associated with FHR in HBeAg-negative patients. HBcrAg is a novel biomarker in CHB patients and consists of the hepatitis B core antigen, HBeAg, and the 22-kDa precore protein.12 It gradually decreased after antiviral therapy. Patients with severe alanine transaminase flares had increased HBcrAg levels after antiviral therapy cessation, and the concentration declined after recommencing antiviral therapy.13

The decline of HBcrAg during antiviral therapy reflects intrahepatic cccDNA reduction and suppression of viral replication activity. HBcrAg decline was positively correlated with HBsAg reduction or HBeAg seroconversion.14 While the HBeAg expression outnumbers the HBcrAg expression in HBeAg-positive patients, the predictive performance of HBcrAg on FHR may be affected. After antiviral treatment cessation, patients with lower HBcrAg levels are more likely to have HBsAg loss (Table 1).15 It may be appropriate to monitor HBcrAg to assess clinical outcomes and treatment effects.

Abbreviations:
- cccDNA: covalently closed circular DNA
- CHB: chronic hepatitis B
- EOFU: end of follow-up
- FHR: favourable HBsAg response
- HBcrAg: hepatitis B core-related antigen
- HBeAg: hepatitis B e antigen
- HBsAg: hepatitis B surface antigen
- HBV: hepatitis B virus
- HCC: hepatocellular carcinoma
- LLOD: lower limit of detection
- NAs: nucleos(t)ide analogues
- pgRNA: pre-genomic RNA

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In addition to being a useful biomarker for monitoring viral replication activity and cccDNA, HBcrAg is of high value in predicting the development of HCC in CHB patients. For treatment-naïve CHB patients, high serum HBcrAg level was associated with the development of HCC. Patients with decreased HBcrAg had lower HCC risk than those with persistently high HBcrAg levels. Serum HBcrAg also had a superior prediction value for predicting the HCC risk than other HBV markers, such as HBV DNA level (Table 1).

HCC risk was not eliminated in antiviral-treated HBeAg-negative patients with high HBcrAg levels. For antiviral-treated CHB patients, among whom HBV DNA and HBsAg levels may not perform well in HCC risk prediction, HBcrAg can predict the incidence of HCC accurately in HBeAg-negative patients with high sensitivity and negative predictive value. It also works in cirrhotic CHB patients. Consistently high on-treatment serum HBcrAg level was associated with a higher HCC incidence, despite prolonged antiviral treatment, in both HBeAg-positive and HBeAg-negative CHB patients (Table 1). The predictive value for post-treatment HCC recurrence of HBcrAg was also demonstrated. Further studies are needed to explore the underlying mechanism of correlation between high HBcrAg and HCC.

### UNANSWERED QUESTIONS

As with all important studies, the study by Mak and colleagues raises a number of interesting questions. For historical reasons, the majority of patients in this study received older generations of NAs, namely lamivudine, adefovir dipivoxil, and telbivudine. The development of drug-resistant mutants and changes in antiviral drugs during follow-up would have affected the association between early changes in viral markers and long-term disease control. Future studies in patients receiving current first-line treatments (entecavir and tenofovir) are needed.

Moreover, we also need to understand the meaning of FHR in this study. A low serum HBsAg level was associated with a lower risk of HCC in patients with low HBV DNA, and the risk was even lower after HBsAg seroclearance. It would be interesting to determine the role of early pgRNA and HBcrAg response in predicting the off-treatment response and prognostication.

Notably, the field of hepatology is currently working towards functional cure of CHB (i.e., HBsAg seroclearance and sustained off-treatment HBV DNA suppression). Since HBsAg seroclearance is rare with the current oral NAs, the current oral NA treatment is unlikely the area where the new virological markers will be applied. Rather, the roles of HBV RNA, HBcrAg, and HBsAg levels in predicting the response to novel direct-acting antivirals and immunological treatments in HBV cure programs are some of the hottest research areas in hepatology.

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Finding</th>
<th>HBcrAg level</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg loss</td>
<td>HBcrAg decline correlated with HBeAg loss</td>
<td>2.3 log U/mL reduction</td>
</tr>
<tr>
<td>HBsAg loss</td>
<td>Lower HBcrAg correlated with higher incidence of HBsAg loss</td>
<td>&lt;2 log U/mL</td>
</tr>
<tr>
<td>HCC</td>
<td>Higher HBcrAg correlated with higher HCC risk in treatment-naïve patients</td>
<td>&gt;2.9 log U/mL</td>
</tr>
<tr>
<td></td>
<td>Higher HBcrAg correlated with higher HCC risk in antiviral-treated patients</td>
<td>≥4.9 log U/mL for HBeAg-positive patients</td>
</tr>
<tr>
<td></td>
<td>Higher HBcrAg correlated with higher post-treatment HCC recurrence</td>
<td>≥4.8 log U/mL</td>
</tr>
</tbody>
</table>

HBcrAg, hepatitis B core-related antigen; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.
In summary, the study by Mak and colleagues was exceptional in exploring the meaning of early changes in novel virological markers in a cohort with very long follow-up. With concerted effort using novel treatments and biomarkers, we are hopeful that the “holy grail” of functional cure for CHB will be achievable in the future.

Authors’ contribution

All the authors were responsible for the interpretation of findings, the drafting, and critical revision of the editorial for important intellectual content. All authors approved the final version of the article.

Conflicts of Interest

Lilian Liang declared that she has no competing interests. Vincent Wong has served as a consultant or advisory committee member for AbbVie, Boehringer Ingelheim, Echosens, Intercept, Inventiva, Novo Nordisk, Pfizer, and TARGET PharmaSolutions; and as a speaker for Abbott, AbbVie, Gilead Sciences, and Novo Nordisk. He has received a research grant from Gilead Sciences, and is a cofounder of Illuminatio Medical Technology Limited. Grace Wong has served as an advisory committee member for Gilead Sciences and Janssen, and as a speaker for Abbott, AbbVie, Ascelitis, Bristol-Myers Squibb, Echosens, Gilead Sciences, Janssen, and Roche. She has also received a research grant from Gilead Sciences. Terry Yip has served as an advisory committee member and a speaker for Gilead Sciences.

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New biomarkers of hepatitis B virus (HBV) infection: HBV RNA and HBV core-related antigen, new kids on the block?

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Keywords: Hepatitis B virus RNA; Hepatitis B virus core-related antigen; Hepatitis B; Biomarkers

Through long-term treatment with nucleos(t)ide analogues (NUC), the prevention of disease progression to end-stage liver disease and reduction in the risk of hepatocellular carcinoma (HCC) is achievable in most patients with chronic hepatitis B (CHB). However, functional cure (hepatitis B surface antigen [HBsAg] seroclearance) is very rarely achievable even with long-term NUC treatment, and the optimal timing of treatment initiation and discontinuation remains debatable.

With recent advances in molecular analysis, several new biomarkers of the hepatitis B virus (HBV) have been identified, including quantification of HBsAg (qHBsAg), HBV RNA, and HBV core-related antigen (HBcrAg). Integration of these biomarkers with the conventional ones, such as the hepatitis B e antigen (HBeAg) test and HBV DNA quantitation, may improve our understanding of the natural history of CHB and its response to antiviral therapy. All of qHBsAg, HBV RNA, and HBcrAg have been proposed as surrogate markers of HBV covalently closed circular DNA (cccDNA) activity. Studies have shown the potential utility of these novel biomarkers in a range of clinical settings, such as monitoring response to antiviral therapy, predicting relapse after treatment cessation or estimating the risk of HCC.

However, HBsAg may be produced from both cccDNA and integrated viral genomes and its correlation with intrahepatic cccDNA is particularly weak in the HBeAg-negative patients. Therefore, attention has shifted to other serum biomarkers such as HBV RNA and core-lated antigen.

In this issue of Mak et al., a new role of HBV pre-genomic (pg) RNA and HBcrAg in predicting favourable HBsAg response (FHR; <100 IU/mL or HBsAg seroclearance) during median 17 years of NUC treatment. For HBeAg-positive patients, serum HBV pgRNA decline at week 4 was significantly greater for patients with FHR compared to non-FHR patients (5.49 vs. 4.32 log copies/mL, respectively). For HBeAg-negative patients, instead of increase in serum HBcrAg from baseline in non-FHR patients, FHR patients had median reduction in HBcrAg at week 4 (increment of 1.75 vs. reduction of 2.98 log U/mL). This may have a significance as a new study that
showed the usefulness of HBV pgRNA and HBcrAg in predicting FHR during NUC treatment. However, its clinical implication is limited because this study cannot address whether early biomarker response can help to predict successful off-NA virological control. Nonetheless, the results of this study could be used as companion diagnostic tests in the clinical trials to develop new novel drugs to induce CHB functional cure.

It is noteworthy that the HBV RNA and HBcrAg were significantly lower in all time points among HBeAg-negative patients compared to HBeAg-positive patients, a finding that is consistent with previous reports. This is especially true for HBcrAg due to the poor detectability in HBeAg-negative patients because HBeAg is part of HBcrAg (which consists of HbcAg, HBeAg and p22cr). Therefore, the analyses were performed in HBeAg-positive and HBeAg-negative patients separately.

Overall, the use of novel biomarkers for HBV infection carries enormous potential, and it is possible that new biomarker-based models, used in combination with traditional ones, will become an integral part of our daily practice in near future as well as in the development of new drugs. The addition of HBV RNA and HBcrAg to our armamentarium as new biomarkers should be embraced and will be beneficial in our efforts to eliminate HBV. However, before using these biomarkers, the assays for these biomarkers should be standardized with their improvement in the detection sensitivity and also availability.

Conflicts of Interest

YS Lim is an advisory board member of Gilead Sciences and receives research funding from Gilead Sciences. No other disclosures are reported.

REFERENCES


Abbreviations:
cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; FHR, favourable HBsAg response; HBcrAg, HBV core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NUC, nucleos(t)ide analogues; pg, pre-genomic; qHBsAg, quantification of HBsAg
Hepatocellular carcinoma incidence is decreasing in Korea but increasing in the very elderly

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Graphical Abstract

• From 2008 to 2018, the incidence of HCC in Korea gradually declined, however, the incidence of HCC patients aged ≥80 years increased significantly (age-standardized incidence rate increased by 0.96% per year).
• In 2028, the number of HCC patients aged ≥80 years will increase to quadruple greater than that in 2008, comprising 21.3% of all HCC patients.
• Investigation in trends of HCC incidence will guide tailored management plan for elderly patients, and will help making a national health strategy to reduce the socioeconomic burden of HCC.

Study Highlights

• From 2008 to 2018, the incidence of HCC in Korea gradually declined, however, the incidence of HCC patients aged ≥80 years increased significantly (age-standardized incidence rate increased by 0.96% per year).
• In 2028, the number of HCC patients aged ≥80 years will increase to quadruple greater than that in 2008, consisting 21.3% of all HCC patients.
• Investigation in trends of HCC incidence will guide tailored management plan for elderly patients, and will help making a national health strategy to reduce the socioeconomic burden of HCC.

The age-standardized incidence rate of patients with HCC aged ≥80 years over the past 10 years in Korea, and the predicted number of HCC patients aged ≥80 years in 2028 will be quadruple greater than the number of HCC patients in 2008, comprising 21.3% of all HCC patients in 2028.
INTRODUCTION

Primary liver cancer is the sixth most common cancer and the third leading cause of cancer-related deaths worldwide. \(^1\,^2\) In 2020, the highest age-standardized incidence rates (ASRs) of liver cancer were observed in Eastern Asia (17.8/100,000 person-years), Northern Africa (15.2/100,000 person-years), South-Eastern Asia (13.7/100,000 person-years), and South Korea (14.3 cases/100,000 person-years). \(^1\) In South Korea, liver cancer ranked second only to lung cancer as a cause of cancer-related deaths in 2016. \(^3\) As liver cancer has the highest mortality rate in middle-aged Koreans (i.e., individuals aged 40–59 years) having the highest socioeconomic productivity, there is a substantial disease burden associated with hepatocellular carcinoma (HCC). Therefore, efforts have been continued to accurately investigate the incidence of liver cancer and to establish proper strategies to reduce liver cancer accordingly.

The incidence of liver cancer has been extensively investigated worldwide, but the incidence of HCC is relatively
known. HCC is the most common primary liver cancer, comprising 75–85% of all primary liver cancers. Most previous studies reported the incidence of all types of cancers in the liver (e.g., intrahepatic cholangiocarcinoma, hepatoblastoma), and thus did not discuss exclusive data about HCC. In addition, the main source of data for previous epidemiologic studies on HCC in Korea was the Korean Primary Liver Cancer Registry. Although the data from this registry are representative of patients with HCC nationwide, the registry does not include all patients with HCC.

Hepatitis B virus (HBV), hepatitis C virus (HCV), and liver cirrhosis are risk factors for HCC. In a cohort study concerning the Korean population conducted from 2003–2005, the most frequently identified etiologies of HCC were HBV (62.2%), HCV (10.4%), and alcohol or an unknown etiology (27.4%). During the past several decades, many efforts have been made to eradicate HBV, the main cause of HCC. A national project has been in place since 1995 to vaccinate young people against HBV, and patients with chronic hepatitis B (CHB) have been eligible to receive reimbursement for the cost of lifelong antiviral treatment since 2010. As a result, HBV infection has decreased dramatically from 8% in the 1990s to 3% in the 2010s in Korea. However, at the same time, there is an increasing incidence of HCC caused by non-alcoholic fatty liver disease (NAFLD) because of recent westernization of the dietary pattern in Korea. The improved control of viral hepatitis and increased prevalence of metabolic liver diseases suggest that the etiology of HCC may be changing.

Therefore, it is necessary to comprehensively analyze trends in HCC incidence and the factors that affect these trends. We aimed to analyze the trends in the incidence rates of HCC over 10 years (2008–2018) and to predict the incidence rate of HCC in Korea for the year 2028. In addition, we aimed to study serial changes in the incidence of HCC according to age groups, economic classes, and etiologies.

**MATERIALS AND METHODS**

**Study design**

This retrospective cohort study used data from the Korean National Health Insurance Service (KNHIS) database. Data were extracted and coded with an encrypted number, in accordance with the institutional disclosure principle after permission had been obtained from the National Health Corporation. This study was approved by the Institutional Review Board of Hanyang University, Seoul, Republic of Korea (2019-07-014).

**Database characteristics**

The Korean government created universal health insurance systems in 1989 and consolidated them into a single system in 2000. The health insurance system is mandatory for all Korean citizens, and 97.2% of the Korean population has been enrolled in the system since 2018. Data are entered into the KNHIS database when Korean clinics or hospitals submit an insurance claim to the National Health Corporation for their medical services to be reimbursed.

**Inclusion and exclusion criteria**

We included patients from the KNHIS database who had been newly diagnosed with HCC (C220 and V193) between January 2008 and December 2018. We excluded patients if any of the following conditions were met: 1) diagnosis with HCC between 2005 and 2006 due to the low reliability of the data from that period; 2) diagnosis with HCC in the 2 years prior to index date; 3) diagnosis with malignancies other than HCC (C codes other than C220 and V193); 4) history of organ transplantation; 5) human immunodeficiency virus infection; or 6) missing patient identification information (age or sex).

**Primary and secondary endpoints**

The primary endpoints of this study were the change in the incidence rate of HCC over the past 10 years (2008–2018) by age groups and the predicted incidence rate of HCC in Korea for 2028. The secondary endpoints were the changes in the HCC incidence rate according to age groups, economic classes, and etiologies of HCC.

**Stratification**

Patients were divided into four age groups: 0–29 years, 30–59 years, 60–79 years, and ≥80 years. The economic classes of the study participants were determined by the premiums of their medical insurance. Medical insurance groups were classified into 20 categories (Q1 to Q20) based on their in-
insurance premiums. The Medicare group was excluded from these categories because patients in this group were exempted from paying the insurance premiums. The 20 categories were grouped into Q1–5, Q6–10, Q11–15, and Q16–20. Patients in the Q16–20 group paid the highest premium, and patients in the Q1–5 group paid the lowest premium.

| Table 1. Baseline characteristics of the study population |
|-----------------|-----------------|
| Variable        | Patients with HCC (n=127,426) |
| Age (years)     | 61.4 (53.0–70.0) |
| Male gender     | 99,767 (78.3) |
| Etiology        |                |
| HBV             | 80,354 (63.0) |
| HCV             | 12,556 (9.9)  |
| Alcohol         | 12,815 (10.1) |
| NAFLD           | 13,183 (10.3) |
| Others          | 8,518 (6.7)   |
| District        |                |
| Seoul           | 23,428 (18.4) |
| Medical insurances |            |
| Medicare        | 8,580 (6.7)  |
| Q1–5            | 23,716 (18.6) |
| Q6–10           | 24,852 (19.5) |
| Q11–15          | 30,223 (23.7) |
| Q16–20          | 40,055 (31.5) |
| Comorbid conditions |            |
| Liver cirrhosis | 96,593 (75.8) |
| Chronic kidney disease | 10,619 (8.3) |
| Diabetes mellitus | 48,182 (37.8) |
| Hypertension    | 63,991 (50.2) |
| Other neoplasms | 6,766 (5.3)  |
| Cardiovascular diseases | 9,272 (7.3) |
| Cerebrovascular diseases | 15,962 (12.5) |

Values are presented as median (interquartile range) or number (%).
The medical insurance group was classified into 20 categories, excluding medicare group. Twenty categories were regrouped into 4 categories. The higher category stands for the higher income. HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease.

<table>
<thead>
<tr>
<th>Table 2. Annual incidence rate and average annual percent change of hepatocellular carcinoma (2008–2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>2008  2009  2010  2011  2012  2013  2014  2015  2016  2017  2018  AAPC  95% CI  P-value</td>
</tr>
<tr>
<td>CR (per 100,000 person-years)</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>22.4  23.9  22.6  23.2  22.0  22.2  21.7  21.2  20.9  20.4  21.0  -0.94  -4.78 to 3.06  &lt;0.001</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>35.0  37.3  36.1  35.2  35.8  34.6  34.7  33.7  33.1  32.0  31.2  -0.92  -4.00 to 2.26  &lt;0.001</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>9.7  10.5  10.2  10.1  10.0  9.9  9.8  9.7  9.6  9.5  9.3  -0.94  -6.70 to 3.18  0.048</td>
</tr>
<tr>
<td>ASR (per 100,000 person-years)</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>17.6  21.9  20.5  19.3  18.0  17.4  16.7  16.1  15.9  15.1  14.3  -3.90  -4.20 to -3.30  &lt;0.001</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>26.9  33.7  31.4  29.6  27.5  25.5  24.0  21.6  20.7  19.8  -4.20  -4.70 to -3.70  &lt;0.001</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>7.7  9.6  8.5  7.6  7.3  7.1  7.0  6.7  6.4  6.2  -3.90  -5.00 to -2.90  0.011</td>
</tr>
</tbody>
</table>

AAPC, average annual percent change; CI, confidence interval; CR, crude incidence rate; ASR, age-standardized incidence rate.
**Definition of HCC**

The operational definition of HCC included patients who had assigned both the International Classification of Diseases (ICD) code of C220 and the rare incurable disease code of V193 (cancer). If a patient is diagnosed with a cancer and assi-

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**Figure 1.** Annual incidence rate of hepatocellular carcinoma (2008–2018) and projections to 2028. (A) Numbers; (B) crude incidence rates (CRs); (C) age-standardized incidence rates (ASRs).
### Table 3. Annual age-standardized incidence rate of hepatocellular carcinoma by age

<table>
<thead>
<tr>
<th>Age</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>AAPC</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (per 100,000 person-years)</strong></td>
<td></td>
<td></td>
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<tr>
<td>All</td>
<td>21.9</td>
<td>20.5</td>
<td>19.3</td>
<td>19.0</td>
<td>17.8</td>
<td>17.2</td>
<td>16.7</td>
<td>16.1</td>
<td>15.9</td>
<td>15.1</td>
<td>14.3</td>
<td>-3.90</td>
<td>-4.20 to -3.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>33.7</td>
<td>31.4</td>
<td>29.6</td>
<td>29.0</td>
<td>27.5</td>
<td>26.4</td>
<td>25.5</td>
<td>24.6</td>
<td>24.0</td>
<td>22.7</td>
<td>21.6</td>
<td>-4.20</td>
<td>-4.70 to -3.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>9.6</td>
<td>9.1</td>
<td>8.5</td>
<td>8.4</td>
<td>7.6</td>
<td>7.3</td>
<td>7.1</td>
<td>7.0</td>
<td>6.7</td>
<td>6.2</td>
<td>6.2</td>
<td>-3.90</td>
<td>-5.00 to -2.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>0–29 years (per 100,000 person-years)</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>All</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-13.59</td>
<td>-16.48 to -10.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-14.10</td>
<td>-19.70 to -8.10</td>
<td>&lt;0.001</td>
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<tr>
<td>Women</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-44.60</td>
<td>-88.80 to 166.20</td>
<td>0.400</td>
</tr>
<tr>
<td><strong>30–59 years (per 100,000 person-years)</strong></td>
<td></td>
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<td>7.70 to 9.90</td>
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AAPC, average annual percent change; CI, confidence interval.
signed the V193 code, the patient only pays 5% to 10% of the total medical expenses. Cancer patients covered 10% of the total cost in 2008 and 2009; the proportion decreased to 5% in December 2009. Therefore, the KNHIS requests more information at the time of HCC diagnosis, and these patients are strictly monitored by the government. The KNHIS reviewed

![Figure 2](https://doi.org/10.3350/cmh.2021.0395)

**Figure 2.** Annual incidence rate of hepatocellular carcinoma (2008–2018) in patients aged ≥80 years. (A) Numbers; (B) crude incidence rates (CRs); (C) age-standardized incidence rates (ASRs).
whether HCC was diagnosed based on histology, dynamic computed tomography, and/or magnetic resonance imaging findings. The imaging findings suggestive of HCC included a liver nodule > 1 cm, arterial hypervascularity of the nodule, and washout in the portal venous or delayed phase.  

**Identification of etiologies and comorbid conditions of HCC**

Etiologic diseases (CHB, chronic hepatitis C, alcoholic liver disease, and NALFD) were identified if the code for the disease or prescription of its treatment was assigned in the calendar year containing the index date or within one calendar year before or after this year (3 years). When two or more etiologic factors were present, patients were classified into the class for the higher-ranked condition in the following order: HBV, HCV, alcoholic liver disease, NAFLD, and others. We have added this information to the Methods section. Comorbid conditions (liver cirrhosis, diabetes mellitus, hypertension, chronic kidney disease, other malignancies, cardiovascular diseases, and cerebrovascular diseases) were identified if the relevant ICD codes were assigned twice during the calendar year containing the index date or within one calendar year before or after this year (3 years). Each definition and the relevant ICD codes were depicted in Supplementary Table 1.

**Statistical analysis**

Data are presented as the median (interquartile range) or frequencies with percentages, as appropriate. The 2005 Korean population covered by the KNHIS was used as the standard population for calculation of the ASR. To evaluate trends in the incidence of HCC during the past 10 years, we used the joinpoint regression method to calculate the average annual percent change (AAPC) in incidence rates. We used the Poisson regression model to predict the incidence of HCC. All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA) and R software (version 3.6.0; http://cran.r-project.org/). Two-sided P-values < 0.05 were considered statistically significant.

![Figure 3. Annual changes in etiologies of hepatocellular carcinoma (2008–2018). HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease.](http://www.e-cmh.org)
RESULTS

Baseline characteristics

We identified 127,426 patients in the KNHIS database who had been newly diagnosed with HCC between 2008 and 2018 (Supplementary Fig. 1). The baseline characteristics of these patients are summarized in Table 1. The mean age at the time of HCC diagnosis was 61 years, and there was a male predominance (78.3%). HBV was the most common etiology of HCC, affecting 63.0% of all patients with HCC. The top quarter of medical insurance groups (Q16–20) had the largest proportion of patients with HCC (31.5%). Liver cirrhosis, hypertension, and diabetes mellitus were present in 75.8%, 50.2%, and 37.8% of patients with HCC, respectively.

Incidence of HCC

We identified 127,426 patients newly diagnosed with HCC between 2008 and 2018 from the KNHIS database, yielding an average annual incidence rate of 11,584 patients. The average crude incidence rate (CR) of HCC incidence was 22.4 per 100,000 person-years (Table 2, Fig. 1), and the average ASR was 17.6 per 100,000 person-years. HCC was more frequent in men than in women, and the age-standardized male-to-female incidence ratio was 3.49 (95% confidence interval [CI], 3.10–3.72; \(P=0.032\)). The annual numbers of HCC incidence significantly decreased from 2008 to 2018 (from 12,056/year in 2008 to 11,234/year in 2018; AAPC: -0.42%; 95% CI, -0.59 to -0.25; \(P<0.001\)). The decline in annual numbers of HCC incidence was observed for both sexes (AAPC in men, -0.43%; AAPC in women, -0.37%). The CR of HCC incidence decreased from 23.9 per 100,000 person-years in 2008 to 21.2 per 100,000 person-years in 2018, but this change was not statistically significant (AAPC, -0.94%; CI, -4.0 to 3.8; \(P=0.640\)). The ASR in the entire population significantly decreased from 2008 to 2018 (from 21.9/100,000 person-years in 2008 to 14.3/100,000 person-years in 2018; AAPC, -3.9%; CI, -4.2 to -3.5; \(P<0.001\)). The decline in ASR between 2008 and 2018 was evident in both men (AAPC, -4.2%; CI, -4.7 to -3.7; \(P<0.001\)) and women (AAPC, -3.9%; CI, -5.0 to -2.9; \(P<0.001\)).
Incidence of HCC by age groups

HCC was diagnosed most frequently in patients aged 60–79 years (46.9%), followed by patients aged 30–59 years (45.9%), ≥80 years (6.9%), and 0–20 years (0.3%). The ASR significantly decreased in patients aged 0–29 years and 30–59 years (Table 3, Supplementary Fig. 2). The decline in annual ASR was greater in patients aged 0–29 years (AAPC, -13.59%; 95% CI, -16.48 to -10.60) than in patients aged 30–59 years (AAPC, -3.10%; 95% CI, -4.10 to -2.10; P<0.001; Table 3, Supplementary Fig. 3). There was a significant decrease in ASR in both sexes in most groups of patients aged <80 years, but patients aged 0–29 women and patients aged 60–79 men did not show significant decrease in ASR. The average annual change was greatest in men aged 0–29 years (AAPC, -14.10%; 95% CI, -19.70 to -8.10; P<0.001). The numbers of HCC and CR significantly decreased in patients aged 0–29 years and 30–59 years (Supplementary Table 2). In patients aged 60–79 years, the numbers of HCC did not change, whereas the CR of HCC incidence significantly decreased. The ASR in patients aged ≥80 years increased from 70.0 per 100,000 person-years in 2008 to 160.2 per 100,000 person-years in 2018 (AAPC, 9.00%; 95% CI, 7.90–10.20; P<0.001; Fig. 2C). This increase was evident in both men (AAPC, 9.40%; 95% CI, 8.42–10.45; P<0.001) and women (AAPC, 8.80%; 95% CI, 7.70–9.90; P<0.001; Table 3). During the 10-year study period, the numbers of HCC increased in patients aged ≥80 years (AAPC, 9.90%; 95% CI, 7.90–10.20; P<0.001; Supplementary Table 2, Fig. 2A). The CR of HCC incidence in patients aged ≥80 years also significantly increased (AAPC, 1.40%; 95% CI, 0.80–1.90; P<0.001; Supplementary Table 2, Fig. 2B).

Incidence of HCC by economic classes

Patients with HCC were diagnosed most frequently in the Q16–20 group (31.5%), followed by the Q11–15 (23.7%), Q6–10 (19.5%), Q6–10 (18.6%), and Medicare (6.7%) groups. However, the ASR was significantly greater in the Medicare group than in the Q16–20 group. From 2008 to 2018, there was a significant decrease in the ASR in all groups (Supplementary Fig. 4, Supplementary Table 3), except the Medicare group. There was no significant change in the ASR in the Medicare group (AAPC, -1.40%; P=0.202).

Figure 4. Annual proportions of hepatocellular carcinoma cases according to age groups (2008–2018) and projection in 2028.
Etiologies of HCC

The most common etiology of HCC was HBV (63.0%), followed by NAFLD (10.4%), alcohol (10.1%), and HCV (9.9%; Fig. 3). The proportion of patients with HBV-related HCC did not significantly change from 2008 to 2018 (P=0.210). However, the proportion of HBV-related HCC significantly decreased between 2011 and 2018 with an AAPC of -1.40% (95% CI, -2.10 to -0.70; P<0.001). The proportion of HCV-related HCC significantly increased with an AAPC of 1.00% (95% CI, 0.20–1.90; P<0.001), contributing to 9.1% of HCC cases in 2008 and 10.0% in 2018. The proportion of alcohol-induced HCC increased from 8.7% in 2008 to 12.3% in 2018 (AAPC, 3.20%; 95% CI, 2.50–3.90; P<0.001). Specifically, the proportion of NAFLD-related HCC steadily increased from 2008 to 2018 (AAPC, 2.70%; 95% CI, 0.20–5.30; P<0.001), with a rapid increase from 2011 to 2018 at an average of 5.60% per year (95% CI, 3.20–8.10; P<0.001).

Projections of HCC incidence to 2028

The observed and predicted trends in HCC incidence among men and women are summarized in Figure 1 and Table 4. We predict that number of new HCC patients in 2028 will be 9,663 (7,264 men; 2,399 women). The CR of HCC incidence in 2028 is estimated to be 17.9 per 100,000 person-years (men: 26.7/100,000 person-years; women: 8.9/100,000 person-years). The predicted ASR will decline by 9.7 per 100,000 person-years in 2028. The ASRs in men and women are expected to be 14.3 and 4.1 per 100,000 person-years, respectively. The annual changes of ASR from 2008 to 2028 are expected to be -3.9% (95% CI, -4.0 to -3.8; P<0.001) for the entire population, -4.1% (95% CI, -4.2 to -4.0; P<0.001) for men, and -4.0% (95% CI, -4.1 to -3.9; P<0.001) for women, respectively. From 2008 to 2028, the ASR is expected to significantly decrease in age groups 0–29 and 30–59 years. The annual decrease of ASR from 2008 to 2028 is expected to be the greatest in the age group 0–29 years with an AAPC of -15.7% (95% CI, -16.6 to -14.8; P<0.001). In 2028, the number of HCC patients aged ≥80 years is estimated to increase quadrupule, compared with the number in 2008 (from 521 in 2008 to 2,055 in 2028). The ASR (from 70.0 to 387.6/100,000 person-years) of HCC incidence of that age is also expected to increase, with an annual increase in ASR of 9.3% (95% CI, 9.2–9.3; P<0.001).

Figure 4 shows the proportions of HCC cases in different age groups (2008–2018) and the projected proportions in 2028. From 2008 to 2028, the proportions of patients with HCC developing at ages 0–29 years (AAPC, -11.3%; 95% CI, -14.5 to -8.0; P<0.001), and 30–59 years (AAPC, -5.2%; 95% CI, -5.5 to -4.8; P<0.001) are estimated to significantly decrease. Conversely, the proportions of HCC patients at ages 60–79 years (AAPC, 1.4%; 95% CI, 1.1–1.8; P<0.001), and ≥80 years (AAPC, 8.5%; 95% CI, 8.0–9.0; P<0.001) are estimated to significantly increase. The proportion of HCC patients aged ≥80 years will increase from 4.3% in 2008 to 21.3% in 2028, and it is estimated that patients older than 60 years will comprise 83.1% in 2028.

DISCUSSION

From 2008 to 2018, the numbers and ASRs of HCC significantly decreased. However, in patients aged ≥80 years, the ASR significantly increased by 0.96% per year; the CR of HCC also increased. By 2028, despite the estimated decrease in HCC incidence in the entire population, the incidence and the proportion of HCC patients aged ≥80 years are estimated to increase. To our knowledge, this study is the first to include a large number of patients (n=127,426) from the KNHIS database, which includes most Korean citizens. The inclusion of such a large number of patients has enabled a comprehensive analysis of the longitudinal trends in HCC incidence in Korea. The estimation of the current and future incidences of HCC in different age groups is important for performing an economic evaluation of the burden of HCC and for formulating an appropriate healthcare policy to control HCC.

There are several important clinical implications of this study. First, the decreasing trend in the ASR of HCC incidence in Korea is similar to the trends observed in Asians living in other countries. A recent study by Petrick et al. estimated the current and future incidences of HCC in the United States. The overall ASR of HCC increased from 2000 to 2012, and the researchers predicted that it would further increase from 2013 to 2030. In contrast, the ASR of HCC in Asians is expected to decrease from 2013 to 2030 by an average of -1.8% each year, and the ASR of HCC in 2030 is estimated to be 15.5 per 100,000 person-years. Another study from the United States reported that the ASR of HCC significantly decreased from 2007 to 2016 at a rate of -2.72% per year in patients with
Asian/Pacific islander ethnicity. Similar to these two studies, the annual decrease in ASR in our study was -3.9% from 2008 to 2018, and the predicted ASR in 2028 is 9.7% per 100,000 person-years. The project decrease in the ASR and number of patients with HCC in Korea may be a result of better control of HBV and HBV-related HCC. Although HBV remained the strongest contributing factor to HCC in 2018, the total number of patients with HCC and the proportion of cases of HBV-related HCC decreased annually from 2011 to 2018. Nationwide efforts have been made to eradicate HBV, the main cause of HCC. HBV vaccination has been a component of the national vaccination program since 1995; more than 95% of infants are currently vaccinated, which has contributed to a low positivity rate (0.2%) for hepatitis B surface antigen in children. In addition, the introduction and expansion of insurance coverage of antiviral agents for HBV have improved the control of HBV replication and prevented cirrhosis-related complications and HCC.

Second, the HCC incidence trends varied noticeably among age groups. While the absolute number of HCC cases in patients aged 0–29 years was very few, the annual decrease in the ASR was the greatest in this age group (AAPC, -13.59%), and the incidence is predicted to approach zero by 2028. From 2008 to 2018, the ASR decreased in patients aged 30–59 years, and it did not change in patients aged 60–79 years. The ASR anticipated to decrease to 15.5/100,000 person-years in patients aged 30–59 years, whereas it is predicted to increase by 103.2 person-years in patients aged 60–79 years by 2028. Despite a gradual decrease in the ASR over time, the socioeconomic burden of HCC is expected to increase because of increasing life expectancy and a greater likelihood of HCC with increasing age in patients with cirrhosis. Therefore, efforts should be made to control the HCC incidence in middle-aged patients with the highest socioeconomic productivity to alleviate the socioeconomic burden.

Third, it is concerning that the ASR of HCC is not declining in patients receiving Medicare. From 2008 to 2018, the ASR significantly decreased in all medical insurance groups, except the Medicare group. Conversely, there was a slight increase in the ASR from 2012 to 2018 in the Medicare group, although this difference was not statistically significant. It is unclear whether economic class directly affects HCC incidence. In a study by Anyiwe et al., patients in the lower income groups had an increased risk of HCC compared with those in the highest income group, despite adjustments for age, sex, and area of residence. Although economic class itself was not a risk factor for HCC, many risk factors for HCC were closely related to economic class. In our study, we did not find any differences in HCC etiology among medical insurance groups (Supplementary Table 4). However, between 2008 and 2018, patients with HBV-related HCC in the Medicare group had significantly less use of antiviral treatment, than patients in the group with the highest medical insurance (Q16–20) (Supplementary Table 5). Further research is needed to confirm whether the reduced use of antiviral drugs in HBV-infected patients in the Medicare group is linked to uncontrolled viremia and thus affects HCC development.

Fourth, the proportions of patients developing HCC due to HBV and HCV still remained high in 2018, while the proportions of patients with HCC induced by NAFLD and alcoholic liver disease are increasing. In 2016, the World Health Organization set a goal of eliminating HBV and HCV by the year 2030 using prevention and treatment targets that aim to reduce annual deaths by 65%, increase the proportion of treated patients to 80%, and save 7.1 million lives globally. Consistent with this 2030 eradication strategy, vaccination against HBV and antiviral treatment for HBV and HCV will further reduce the incidence of HCC. However, the proportion of patients with NAFLD-related HCC increased by 2.7% annually in our study. NAFLD is the fastest growing cause of HCC in the United States and United Kingdom. The prevalence of NAFLD-related HCC is likely to increase concomitantly with the increasing incidence of obesity. Therefore, management of metabolic indices through lifestyle modification is important for reducing the risk of HCC.

Finally, special attention is needed for patients with HCC aged ≥80 years. The absolute number, CR, and ASR of HCC in patients aged ≥80 years has significantly increased from 2008 to 2018, and the incidence is expected to continue increasing until 2028. An increasing trend in the ASR in the elderly population was also identified in a Taiwanese cohort, and the most plausible explanation was that old age is a risk factor for HCC. The increasing incidence of HCC in older age groups is similar to that seen in other cancers. According to annual statistics from the Korean Central Cancer Registry, the proportion patients with gastric cancer aged ≥80 years was 7.5% and 12.7% in 2008 and 2018, respectively. The proportion patients with colon cancer aged ≥80 years was 9.4% in 2008 and 19.0% in 2018. Moreover, elderly patients have many comorbid conditions, such as liver cirrhosis or diabetes.
mellitus, which may further increase the risk of HCC. In our subgroup analysis of patients with HBV-related HCC, patients aged ≥80 years received significantly less antiviral treatment between 2008 and 2018 than did patients in other age groups (P<0.001; data not shown). HBV is the most common etiology of HCC; therefore, uncontrolled HBV viremia in elderly patients can be associated with a greater incidence of HCC. This assumption requires subsequent verification in future studies. By 2028, the proportion of patients with HCC aged ≥80 years will increase to 21.3% of all patients with HCC. This is consistent with the report by Kim and Park⁴ that the age distribution of HCC in Korea has shifted towards the right between 2005 and 2014. When we look up the age structure of general population, the number of Koreans aged 80 years or older increased from 2008 to 2018 (number, 890,000 in 2008 to 1,845,000 in 2018; the proportion, 1.8% in 2008 to 3.1% in 2018).²⁶ Therefore, even considering that Korea is becoming an aging society, the proportion of HCC patients aged 80 years or older increased at a faster rate (4.3% in 2008 to 10.6% in 2018). Elderly patients with HCC often have additional comorbid conditions such as diabetes, hypertension, and renal failure; thus, they may not receive adequate treatment because healthcare professionals are concerned about the abilities of these patients to tolerate standard HCC treatment. Conversely, using standard treatment for HCC without considering the frailty of these patients may cause toxicity or fatality. Therefore, guidelines should be formulated for customized management of elderly patients with HCC to improve the treatment and reduce the socioeconomic burden of HCC.

There were several limitations in our study. First, although we included the entire dataset from the KNHIS database in our analysis, the HCC incidence may have been underestimated because some patients with HCC may not have visited any hospital or the disease codes may have been missed. Second, we could not confirm the medical records of included patients from the hospitals; thus, there was a possibility of misclassification or under-/overestimation of HCC, etiologies of HCC, or comorbid conditions. Third, the operational definition of NAFLD-related HCC was somewhat liberal because we included patients with diabetes mellitus and without any other chronic liver diseases. However, NAFLD-related HCC accounted for only 10% of all HCC cases in our study, and this proportion was similar to a previous Korean study about the etiological distribution of HCC.¹³ Fourth, since we could not perform analysis by birth cohort, it was difficult to determine the exact timing of HCC reduction affected by expansion of HBV vaccination or antiviral treatment for HBV and HCV. Finally, prediction of future incidence assumes that past trends will continue; predictions may be inaccurate if there are various changes in the medical environment.

In conclusion, the ASR of HCC in Korea gradually declined over the past 10 years. However, the number, CR, and ASR of HCC are increasing in patients aged ≥80 years. In 2028, the number of HCC patients aged ≥80 years will be quadruple greater than the number of HCC patients in 2008, and 21.3% of all patients with HCC will be in this age group. A customized HCC management plan that considers the age and general health status of these patients is necessary. In addition, a national health strategy should be implemented to manage the economic burden of HCC.

Authors’ contribution

Soung Won Jeong and Dae Won Jun take responsibility for the integrity of the work, from inception to the published article.

Specific author contributions: Conception: Young Eun Chon, Soung Won Jeong, and Dae Won Jun; Study design: Young Eun Chon, Dae Won Jun, and Soung Won Jeong; Contribution to data acquisition: Young Eun Chon, Seong Yong Park, Eileen Yoon, Soon Sun Kim, Sang Bong Ahn, Soung Won Jeong, and Dae Won Jun; Statistical analysis: Young Eun Chon, Seong Yong Park, Han Pyo Hong, Donghee Son, Jonghyun Lee, Soung Won Jeong, and Dae Won Jun; Writing papers: Young Eun Chon, Soung Won Jeong, and Dae Won Jun.

All authors have reviewed the paper and approved the final version.

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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

Baveno-VII criteria to predict decompensation and initiate non-selective beta-blocker in compensated advanced chronic liver disease patients

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Study Highlights

• Baveno-VII criteria can rule in or rule out CSPH among cACLD patients at baseline.
• Baveno-VII criteria have not been validated to predict decompensation and the need for NSBB in cACLD patients.
• The prevalence of CSPH among cACLD patients remained unclear.
• One-third of cACLD patients fulfilled the non-invasive criteria of CSPH.
• While non-invasive assessment of CSPH predicts decompensation risk and the need for NSBB in cACLD patients, “probable CSPH” is suboptimal to predict decompensation risk in cACLD patients.
• CSPH exclusion criteria might be used to stop NSBB in cACLD patients however further validations are required.

Graphical Abstract

Baveno-VII criteria to predict decompensation in 1,159 cACLD

Cumulative incidence (%) of liver decompensation, liver-related events, and death over follow-up time in cACLD patients with and without CSPH.
INTRODUCTION

Compensated advanced chronic liver disease (cACLD) patients can be risk-stratified based on the presence of clinically significant portal hypertension (CSPH), which is defined as hepatic venous pressure gradient (HVPG) measurement beyond 10 mmHg. In a randomized trial, Villanueva et al. showed that non-selective beta-blockers (NSBBs) prevent decompensation and improve survival in cACLD patients with HVPG ≥10 mmHg. Although the Baveno-VII consensus recommends NSBB for cACLD patients with CSPH, controversies remain, especially among virologically-suppressed cACLD patients, where the decompensation risk is generally low.

Given the invasive nature and logistic challenges to measuring HVPG in every cACLD patient, the non-invasive assessment of CSPH is an important unmet need. A unifying, non-invasive diagnosis for CSPH was lacking until the recent Baveno-VII consensus. While the combination of baseline liver stiffness measurement (LSM) and platelet count (Bave-
no-VII criteria) correlates with baseline CSPH, this proposed non-invasive assessment of CSPH has not been validated to predict liver decompensation. In this study, we aimed to validate the performance of the Baveno-VII criteria of CSPH to predict liver decompensation and the need for NSBB in an international real-world cohort of cACLD patients.

MATERIALS AND METHODS

This is a retrospective analysis of cACLD patients identified via institutional registries of cACLD patients from four countries (Italy, India, China, and Singapore) between January 2014 and December 2017. We identified cACLD patients based on institutional transient elastography database from Singapore, India, and China, regardless of cirrhosis etiology. The Italy cohort included consecutively treated HCV patients with available transient elastography results consistent with the diagnosis of cACLD (LSM ≥10 kPa). The study was approved by the respective institutional ethics committees with waiver of consent granted, and conducted in compliance with the 1975 Helsinki declaration.

The diagnosis of cACLD was made based on LSM ≥10 kPa with supportive features of cirrhosis such as 1) radiological (nodular liver or irregular liver margin or splenomegaly), 2) histological features of advanced fibrosis or established cirrhosis, 3) presence of gastroesophageal varices or 4) HVPG >5 mmHg. Individual chart review was performed for all patients to confirm the diagnosis of cACLD and relevant clinical data were collected using a unified data template. We excluded patients with a history of liver decompensating events such as ascites, variceal bleeding or hepatic encephalopathy, baseline hepatocellular carcinoma (HCC), invalid LSM, or missing data. Given that treatment of virus-related cirrhosis is the standard of care for cirrhosis patients, we excluded patients with untreated virus-related cirrhosis, which was defined as hepatitis B virus-related cirrhosis without virological suppression or hepatitis C virus-related cirrhosis without sustained virological response. We also excluded patients with significant alcohol intake (30 g/day in males or 20 g/day in females) identified based on electronic medical records. Given that there is no approved specific treatment for non-alcoholic steatohepatitis (NASH) cirrhosis, we included all NASH cirrhosis in our cohort. Finally, we excluded patients with NSBB usage because NSBB use can reduce decompensating events in compensated cirrhosis patients with CSPH, as shown in the PREDESCI (β blockers to Prevent Decompensation of Cirrhosis in Patients with Clinically Significant Portal Hypertension) trial. Given that our study period predated the recent Baveno-VII consensus which recommended the widespread use of empiric NSBB to prevent decompensation in cACLD patients, the treatment of high-risk varices (HRV) was intended to prevent variceal bleeding (rather than decompensation). The decisions between endoscopic variceal ligation versus NSBB were physician-dependent.

LSM

All transient elastography were performed by certified operators using either M or XL probe, based on the manufacturer’s instruction. LSM was measured as the median of at least 10 successful measurements, expressed in kilopascal (kPa). LSM was considered unreliable when the interquartile range (IQR) was beyond 30% of the median LSM value, or when there were less than 10 successful measurements.

Definition of CSPH

We used the Baveno-VII criteria to define CSPH (LSM ≥25 kPa) and exclude CSPH (LSM <15 kPa and platelet ≥150×10⁹/L). Patients who did not fulfill the inclusion or exclusion CSPH criteria were classified as grey zone. Patients within the grey zone were further categorized into high probability of CSPH (defined as LSM between 20–25 kPa and platelet <150×10⁹/L, or LSM between 15–20 kPa and platelet <110×10⁹/L), or low probability of CSPH (defined as the remaining patients within the grey zone).

Study outcomes

Patients were followed-up every 3 to 6 months from the diagnosis of cACLD to the onset of first liver decompensation (variceal bleeding, clinically overt ascites and overt hepatic encephalopathy), HCC or death, whichever occurred earlier. Variceal bleeding was confirmed from the endoscopy. Ascites was defined as clinically overt ascites requiring diuretic treatment. Overt hepatic encephalopathy was defined by West Haven Classification grade 2 and beyond. We defined liver-related events as the presence of either liver decompensation, HCC, or death.
Statistical analysis

Baseline data were summarized based on CSPH criteria into three categories, namely CSPH excluded, grey zone and CSPH (LSM ≥25 kPa). Continuous data were reported in mean±standard deviation or median with IQR based on normality of data distribution. Categorical data were summarized by frequency (percentage). Numerical baseline variables comparisons across the three groups were performed using the one-way analysis of variance/Kruskal-Wallis rank test and chi-square/Fisher’s exact tests for categorical variables. The log-rank test was used to compare the median follow-up times of the three groups.

The risk of liver decompensation was estimated using the competing risk regression for clustered data, with HCC and death as competing risks. The corresponding subdistribution hazard ratio (sHR), 95% confidence interval (95% CI) and cumulative incidence were reported. Cumulative incidences of liver-related events and death were obtained by survival analysis. Subgroup analysis was performed to determine if the presence of HRV and etiology influenced the performance of CSPH to predict decompensation, liver-related events and death among cACLD patients.

Univariable and multivariable competing risk regression for clustered data were conducted to select predictors of liver decompensation regarding HCC and death as competing events. Optimal cut-offs of continuous predictors in the final model were chosen based on the Youden and Liu criteria. All statistical tests were two-sided with a 5% significance level. Statistical analysis was performed using STATA/SE version 17.0 (StataCorp LLC, College Station, TX, USA). Decision curve analysis was performed by R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

Decision curve analysis (DCA)

DCA was used to assess the application of various screening strategies to stratify patients for NSBB to prevent decompensation in real-life settings. DCA evaluates various screening strategies including (1) treating only HRV, (2) treating all esophageal varices (given that varices are manifestations of CSPH), and (3) treating CSPH (diagnosed based on the Baveno-VII non-invasive criteria), in comparison with default strategies of either treating everyone with NSBB, or treating no patients with NSBB. The net benefit of each strategy was assessed across a range of threshold probabilities, with the area under the curve corresponding to the estimated benefit of each strategy to prevent decompensation. Overall, DCA al-

Figure 1. Consolidated Standards of Reporting Trials diagram. cACLD, compensated advanced chronic liver disease; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; NSBB, non-selective beta-blocker; CSPH, clinically significant portal hypertension.
Table 1. Baseline demographic of study subjects stratified based on the non-invasive diagnosis of clinically significant portal hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cohort (n=1,159)</th>
<th>CSPH excluded (n=140)</th>
<th>Grey zone (n=592)</th>
<th>CSPH fulfilled (n=427)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55±13</td>
<td>57±14</td>
<td>57±14</td>
<td>53±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, male</td>
<td>776 (67.0)</td>
<td>89 (63.6)</td>
<td>374 (63.2)</td>
<td>313 (73.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>357 (30.8)</td>
<td>66 (47.1)</td>
<td>207 (35.0)</td>
<td>84 (19.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chinese</td>
<td>328 (28.3)</td>
<td>47 (33.6)</td>
<td>165 (27.9)</td>
<td>116 (27.2)</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>310 (26.8)</td>
<td>2 (1.4)</td>
<td>128 (21.6)</td>
<td>180 (42.2)</td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>111 (9.6)</td>
<td>20 (14.3)</td>
<td>62 (10.5)</td>
<td>29 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Arabic</td>
<td>28 (2.4)</td>
<td>2 (1.4)</td>
<td>18 (3.0)</td>
<td>8 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>25 (2.2)</td>
<td>3 (2.1)</td>
<td>12 (2.0)</td>
<td>10 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>247 (21.3)</td>
<td>34 (24.3)</td>
<td>127 (21.5)</td>
<td>86 (20.1)</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>650 (56.1)</td>
<td>95 (67.9)</td>
<td>374 (63.2)</td>
<td>181 (42.4)</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>105 (9.1)</td>
<td>1 (0.7)</td>
<td>28 (4.7)</td>
<td>76 (17.8)</td>
<td></td>
</tr>
<tr>
<td>NASH</td>
<td>102 (8.8)</td>
<td>4 (2.9)</td>
<td>41 (6.9)</td>
<td>57 (13.4)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>55 (4.7)</td>
<td>6 (4.3)</td>
<td>22 (3.7)</td>
<td>27 (6.3)</td>
<td></td>
</tr>
<tr>
<td>MELD score</td>
<td>8±3</td>
<td>7±1</td>
<td>8±3</td>
<td>9±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Child-Turcott-Pugh score</td>
<td>5.2±0.6</td>
<td>5.0±0.2</td>
<td>5.1±0.4</td>
<td>5.4±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LSM (kPa)</td>
<td>23.8±12.2</td>
<td>12.3±1.3</td>
<td>17.9±3.7</td>
<td>35.7±12.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis-4</td>
<td>4.4±3.6</td>
<td>2.1±1.1</td>
<td>4.4±3.4</td>
<td>5.3±4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>40±5</td>
<td>43±4</td>
<td>41±5</td>
<td>38±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>19±15</td>
<td>14±8</td>
<td>17±11</td>
<td>24±19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (μmol/L)</td>
<td>77±63</td>
<td>75±62</td>
<td>79±65</td>
<td>73±62</td>
<td>0.368</td>
</tr>
<tr>
<td>Platelets (×10^3/μL)</td>
<td>141±66</td>
<td>205±49</td>
<td>138±64</td>
<td>125±62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count (×10^3/μL)</td>
<td>&lt;150</td>
<td>708 (61.1)</td>
<td>0 (0.0)</td>
<td>408 (68.9)</td>
<td>300 (70.3)</td>
</tr>
<tr>
<td>INR</td>
<td>≥150</td>
<td>451 (38.9)</td>
<td>140 (100.0)</td>
<td>184 (31.1)</td>
<td>127 (29.7)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>1.09±0.14</td>
<td>1.03±0.08</td>
<td>1.07±0.13</td>
<td>1.14±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Esophageal varices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No varices</td>
<td>641 (60.7)</td>
<td>88 (82.2)</td>
<td>367 (69.0)</td>
<td>186 (44.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low-risk varices</td>
<td>357 (33.8)</td>
<td>19 (17.8)</td>
<td>140 (26.3)</td>
<td>198 (47.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-risk varices</td>
<td>58 (5.5)</td>
<td>0 (0.0)</td>
<td>25 (4.7)</td>
<td>33 (7.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Follow-up time (months)</td>
<td>40 (30–52)</td>
<td>44 (34–53)</td>
<td>40 (31–52)</td>
<td>39 (30–50)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation, median (interquartile range), or frequency (%).
CSPH, clinically significant portal hypertension; MELD, Model of End-stage Liver Disease; LSM, liver stiffness measurement; ALT, alanine aminotransferase; INR, international normalized ratio.
ows objective assessment of the number of additional patients experiencing decompensation for every patient treated with NSBB using various screening strategies. Further details of DCA are described in Supplementary Material 1.

### RESULTS

#### Baseline characteristics

A total of 1,159 cACLD patients were included and the Consolidated Standards of Reporting Trials diagram was summarized in Figure 1. The cohort was predominantly male with virus-related cirrhosis (77.4%) with Child-Turcott-Pugh (CTP)

![Figure 2](image-url) Clinical outcomes according to the non-invasive diagnosis of clinically significant portal hypertension in compensated advanced chronic liver disease patients. Liver decompensation was defined as the presence of ascites, variceal bleeding and hepatic encephalopathy. Liver-related events was defined as the presence of liver decompensation, hepatocellular carcinoma or death. CSPH, clinically significant portal hypertension; sHR, subdistribution hazard ratio; CI, confidence interval; NA, not applicable.

**Table 2.** Cumulative incidence of liver-related events stratified based on non-invasively assessed clinically significant portal hypertension status

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of events (cumulative incidence %) at 3-year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver decompensation*</td>
</tr>
<tr>
<td>CSPH excluded (n=140)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Grey-zone (n=592)</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>Low probability of CSPH</td>
<td>8 (3.7)</td>
</tr>
<tr>
<td>High probability of CSPH</td>
<td>59 (13.8)</td>
</tr>
</tbody>
</table>

Definition of CSPH category: CSPH, defined as liver stiffness measurement (LSM) ≥25 kPa; CSPH excluded, defined as LSM <15 kPa and platelet count ≥150; grey-zone, patients who did not fulfilled non-invasive criteria to diagnose or exclude CSPH; high probability of CSPH, defined as LSM 20–25 kPa & platelet count <150, or LSM 15–20 kPa & platelet count <110×10⁹/L; low probability of CSPH, other patients within the grey zone.

CSPH, clinically significant portal hypertension.

*Cumulative incidence was calculated based on competing risks regression for clustered data with hepatocellular carcinoma and death as competing risks.

†Cumulative incidence was calculated based on Cox regression with shared frailty.
class A (95.0%); 10.3% had body mass index >30 kg/m². Baveno-VII criteria stratified cACLD patients into three categories, namely CSPH (36.8%), grey zone (51.1%) and CSPH excluded (12.1%). Patients with CSPH had more advanced liver disease (higher CTP score, higher Model of End-stage Liver Disease score, higher bilirubin, lower albumin and lower platelet count), higher mean LSM and Fibrosis-4 score than those without CSPH (P<0.001 for all) (Table 1). The details of each cohort stratified by study sites were summarized in Supplementary Table 1.

Non-invasive diagnosis of CSPH and decompensation

Over a median (IQR) follow-up of 40 months (30–52), 83 patients (7.2%) developed liver decompensation, 67 patients (5.8%) had de-novo HCC, 51 patients (4.4%) died, and none received liver transplantation. The commonest decompensating event was ascites, followed by variceal bleeding and hepatic encephalopathy (Supplementary Table 2). None of the 140 patients (12.1%) fulfilling the exclusion criteria for CSPH developed liver decompensation.

CSPH patients had a higher risk of liver decompensation, liver-related events and death when compared to those without CSPH (Fig. 2, Supplementary Table 3). The risk of liver decompensation was low among patients within the grey zone, regardless of whether they had a high or low probability of CSPH based on non-invasive criteria (Table 2). After excluding subjects with HRVs, these findings remained the same (Supplementary Fig. 1).

Predictors of liver decompensation were the presence of CSPH (sHR, 2.48 [1.35–4.55]; P=0.003), non-viral related cirrhosis (sHR, 3.25 [1.83–5.76]; P<0.001), international normalized ratio (INR) > 1.1 (sHR, 2.08 [1.14–3.80]; P=0.017), and albumin <37 g/L (sHR, 3.38 [1.83–6.25]; P<0.001) (Supplementary Table 4). Application of the “Rule-of-five” in our cohort demonstrate an incremental risk of liver decompensation, with LSM >25 kPa significantly associated with a higher risk of liver decompensation (Supplementary Table 5).

Subgroup analysis by etiology

Given that non-virus-related cirrhosis has a higher risk of decompensation, we performed a subgroup analysis based on etiology. The exclusion criteria of CSPH performed well in excluding patients at risk of decompensation, regardless of the underlying etiology of cirrhosis. Similarly, the presence of CSPH also predicts a higher risk of decompensation com-

http://www.e-cmh.org

https://doi.org/10.3350/cmh.2022.0181
pared to those with CSPH excluded (Fig. 3). The 3-year cumulative incidence of decompensation among non-virus-related cACLD (CSPH excluded, 0%; low probability, 15.0%; high probability, 14.3%; CSPH, 22.2%) was higher than virus-related cACLD patients (CSPH excluded, 0%; low probability, 0.3%; high probability, 1.8%; CSPH, 9.0%).

While the decompensation risk between the grey zone and those with CSPH excluded were similar among virus-related cACLD patients, such risk was substantially higher among non-viral cACLD patients within the grey zone. Among cACLD patients within the grey zone, there were no differences observed between patients with a low or high probability of CSPH, regardless of the underlying etiology (Fig. 3). The predictors of decompensation among patients in grey zone were the etiology of liver cirrhosis, INR, albumin and bilirubin (Supplementary Table 6).

Decision curve analysis

Using the treatment threshold derived from our cohort (between 5–10% decompensation rate at 5 years), “treating definite CSPH” strategy is superior to “treating probable CSPH” and “treat any varices” strategy to initiate NSBB. This is demonstrated by the largest area under the curve by adopting “treating definite CSPH” strategy. The number needed to treat for CSPH-based strategy was 27 and 50, at treatment thresholds of 5% and 10%, respectively (Fig. 4, Supplementary Table 7).

DISCUSSION

This international multicenter study demonstrates that non-invasive assessment of CSPH predicted liver decompensation in a large cohort of cACLD patients. Baveno-VII criteria reliably exclude CSPH, complementing earlier study by Ripoll et al. showing a negligible risk of liver decompensation in patients with HVPG below 10 mmHg. CSPH was present in one-third of cACLD patients, and was associated with a five-fold higher risk of liver decompensation. Moreover, our DCA further supports the strategy of initiating NSBB in patients with CSPH to prevent liver decompensation, which is in line with an earlier randomized trial supporting the use of carvedilol in preventing liver decompensation.

Compared to the seminal PREDESCI study, our cohort had a lower 5-year decompensation risk than the seminal PREDESCI study (7.9% vs. 20%, P<0.001) because the majority of the HCV-related cirrhosis patients from the PREDESCI cohort
were untreated, and only 37% of our cohort had CSPH. While one may argue that virological suppression would have modified the natural course of CSPH in virus-related cirrhosis, our findings reflect the real-world setting where virological suppression is now an achievable standard of care in virus-related cACLD patients following the introduction of high-efficacious, panogenotypic direct-acting antiviral treatment. Moreover, our decompensation rate was consistent with recent studies demonstrating a relatively low risk of decompensation among virologically-suppressed cACLD patients.

Despite the heterogeneous risk of CSPH, we found the risk of liver decompensation in patients within the CSPH grey zone remained similar, irrespective of the underlying etiology of cirrhosis. The Baveno-VII consensus defined patients within CSPH “grey zone” as having either LSM between 20–25 kPa and platelet <150×10^9/L (defined as high probability of CSPH in our study), or LSM between 15–20 kPa and platelet <110×10^9/L (defined as low probability of CSPH in our study). While these patients had “at least” 60% predicted risk of CSPH, the observed risk of CSPH within the ANTICIPATE Cohort ranges from 57% to 78.5% in cACLD patients with low or high probability of CSPH, respectively. In other words, the diagnosis of CSPH grey zone may not be accurate in up to 43%. This heterogeneity in baseline decompensation risks can influence the treatment magnitude of NSBB in terms of absolute risk reduction and number needed to treat, thus making the diagnosis of “CSPH grey zone” unfavourable to be used as selection criteria to initiate NSBB in cACLD patients. In our cohort, the etiology of liver cirrhosis, serum INR, albumin and bilirubin correlates with the risk of liver decompensation in patients within CSPH grey zone (Supplementary Table 6). A recent study by Dajti and colleagues demonstrated that spleen stiffness can reduce the proportion of patients within the CSPH grey zone. Further studies are required to validate the performance of spleen stiffness to stratify decompensation risk in patients with the CSPH grey zone.

Unlike virus-related cACLD whereby only patients with CSPH are at a higher risk of decompensation, a higher risk of decompensation was observed in non-virus-related cACLD patients with CSPH, as well as those within the grey zone. The exact reasons cannot be elucidated due to the relatively small proportion of non-virus-related cACLD in the current study. However there were several postulations: 1) the lack of definitive treatment for NASH cirrhosis may predispose these patients to an increased risk of disease progression and decompensation, 2) the potential difference in the natural history between NASH and virus-related cACLD, where the former may experience clinical decompensation at a lower portal pressure, and 3) unreported ongoing alcohol drinking, which may contribute to a higher risk of decompensation among alcoholic liver cirrhosis. Given the risk of decompensation is substantially higher in non-virus-related cACLD patients within the grey zone, one should remember that the decompensation risk is a continuous spectrum, therefore over-reliance on specific cut-off may potentially oversimplify the risk prediction in cACLD patients.

The expanding treatment indication of NSBB from preventing variceal bleeding to preventing decompensation represents a paradigm shift in the management of cACLD patients. While the Baveno-VI criteria could safely reduce the need for screening gastroscopy, it was unclear if this may represent a missing opportunity to identify cACLD patients with small esophageal varices for NSBB. While the risk of variceal bleeding is generally small in these patients, we demonstrated that the presence of small esophageal varices was associated with a higher risk of liver decompensation in our previous study. In this regard, decision curve analysis showed that the “treating all esophageal varices” strategy is superior to “treating only HRV” strategy to prevent decompensation. However, even with routine endoscopy, CSPH may be present in those without esophageal varices. Indeed, decision curve analysis showed that the best strategy to prevent decompensation is not treating “probable CSPH” or “treating all esophageal varices”, but “treating definite CSPH strategy” instead (Supplementary Table 7). The strategy of treating definite CSPH strategy may prevent more decompensating events, at the same time mitigate the need for routine screening endoscopy among cACLD patients. In other words, the “treat definite CSPH” strategy may have significant impacts on resource allocation and carbon emission, given that endoscopy is the third-largest generator of carbon emission within healthcare.

The strengths of our study include the multicentre design with a relatively large sample size. We validated the performance of non-invasive Baveno-VII criteria using the competing risk analysis to account for multiple competing outcomes among cirrhosis patients, where the occurrence of an event could preclude (death) or modify (HCC or non-hepatic comorbidities) the probability of liver decompensation. Our
findings provide a pragmatic estimation of the decompensation risk, given that most virus-related cirrhosis patients would have been treated with antiviral in a real-world setting. Given the negligible risk of decompensation, NSBB is unlikely beneficial in patients whose CSPH were excluded based on Baveno-VII criteria. Prospective validation will help to understand if this criterion may be used to withdraw NSBB in cACLD patients with primary etiology controlled.

Our study has limitations. Due to the retrospective nature of our study, it is not possible to ensure alcohol abstinence in all subjects as we lack objective tests such as phosphatidylethanol to assess for alcohol intake. While the non-viral etiology was identified as one driving factor of liver decompensation, controlled or cured etiology may likely have contributed to a higher decompensation risk in alcohol-related cACLD (9.1%) as compared to virus-related cACLD patients. We acknowledged that variceal ligation may reduce the incidence of variceal bleeding in patients with HRVs. However, variceal ligation should not influence the incidence of other decompensating events such as ascites or hepatic encephalopathy, and our findings remained consistent after excluding subjects with HRVs (Supplementary Fig. 1). We did not account for radiological evidence of CSPH, such as portosystemic shunts, which may potentially underestimate the prevalence of CSPH in this cohort. We acknowledge that there is variability in patients' characteristics and clinical practice across different institutions, which may potentially influence our results. Our findings remained robust for all key outcomes (liver decompensation, liver-related events and death) after adjusting our analysis by clusters (Fig. 2, Supplementary Table 7). Finally, the application of our findings should also consider the confounders of LSM (obesity, liver congestion or operator experience) and adverse effects of NSBB.

In summary, the non-invasive assessment of CSPH predicts a 5-fold higher decompensation risk in cACLD patients. Our findings support the use of Baveno-VII criteria of CSPH (i.e., LSM >25 kPa) to initiate NSBB in cACLD patients. While the risk of decompensation is low among virus-related cACLD fulfilling exclusion criteria of CSPH (LSM <15 kPa and platelet count ≥150), HCC surveillance should still be continued in these patients. Future studies should focus on identifying disease-specific thresholds to rule in or rule out CSPH following primary etiology suppression.

Authors’ contribution
Study conception: WYJ; Data acquisition: WYJ, SS, GT, ED, LC, LJ; Data analysis: WYJ, CZ, SS, SA, GT, CYH; Manuscript draft: WYJ, SS, SA, GT; Critical review of the manuscript and final review: All authors

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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


Hepatitis B virus pre-genomic RNA and hepatitis B core-related antigen reductions at week 4 predict favourable hepatitis B surface antigen response upon long-term nucleos(t)ide analogue in chronic hepatitis B

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Graphical Abstract

**Study Highlights**

- Early on-treatment HBcrAg and HBV pgRNA showed differential rate of decline between those who achieved favourable HBsAg suppression and those who did not.
- Among those who achieved HBsAg seroclearance or low HBsAg after on antiviral therapy >10 years, week 4 HBV pgRNA or HBcrAg decline were strong predictors (AUROC 0.825 and 0.789, respectively).
- Intrahepatic cccDNA or total HBV DNA decline were not associated with favourable HBsAg suppression.
- Serum pgRNA and HBcrAg allowed the prediction of indefinite duration of therapy in patients put on NAs who should be prioritized for enrolment into clinical trials.
INTRODUCTION

Despite the availability of an effective vaccine, hepatitis B virus (HBV) infection is still a global problem, with an estimate of 292 million people being infected. Currently approved antiviral treatment against chronic hepatitis B (CHB), in the form of nucleos(t)ide analogues (NAs), is very effective in suppression of serum HBV DNA, thereby normalization of liver functions, histological improvement, and reduction in risk of hepatocellular carcinoma and mortality. However, a more desirable treatment endpoint i.e., functional cure, defined as hepatitis B surface antigen (HBsAg) seroclearance, is still a rare event (about 1% per year) even with long-term NA treatment. Moreover, there is a high rate of virological re-
lapse if NA is stopped before achieving functional cure. Therefore, most patients need to take NA on a long-term basis. Little is known about which patients can achieve functional cure so that NA can be stopped after a finite duration. A number of HBV serum markers have been shown to be suppressed by NA and are associated with post-NA cessation virological control. The most-studied markers are HBsAg level, hepatitis B core-related antigen (HBcrAg) and HBV pre-genomic RNA (pgRNA). According to several natural history of disease studies, patients with HBsAg levels <100 IU/mL had a good chance of subsequent loss of HBsAg upon continuation of follow-up. However, the rate of HBsAg decline on NA is modest (0.107 log IU/mL per year) and it will take decades to reach the preferable target of HBsAg level lower than 100 IU/mL.

HBcrAg is a composite measure of three HBV proteins, namely the hepatitis B core antigen, hepatitis B e antigen (HBeAg) and a 22 kDa precore protein. HBcrAg has been shown to correlate with covalently closed circular DNA (cccDNA) levels, and is suppressed by NA treatment. Serum HBV pgRNA levels correlate with the levels and transcriptional activity of cccDNA, and is suppressed by NA therapy. It is not known whether the effect of long-term NA-treatment on HBsAg suppression could be reflected by the early on-treatment changes of HBV serum markers such as HBV pgRNA and HBcrAg.

Therefore, in the present study, we primarily aimed to study the dynamic changes of serum HBcrAg, HBV pgRNA and other serum markers in the first year of NA treatment and investigate whether they can predict favourable HBsAg response (FHR) in the long run. The secondary aim included the investigation of whether serum HBV markers could reflect intrahepatic viral replicative activity.

MATERIALS AND METHODS

Patients

Between January 2002 and April 2009, 215 CHB patients had taken part in three international phase III, randomized, double blind trials in our centre, namely the BEHoLD trials, comparing between entecavir and lamivudine; and the QUASH trial, comparing between clevudine and adefovir. Among them, 124 patients had available paired liver biopsies taken at baseline and year 1 of treatment in our centre. Of these, 67 had available stored serum samples (stored at -20°C) collected at baseline, week 4, week 12, week 24, week 36, and week 48 for analysis in the present study. Of these 67 patients, three were lost to long-term follow-up, leaving 64 patients for final analysis. The primary outcome of this study was the predictors for FHR at end of follow-up (EOFU) upon long-term NA. FHR was defined as HBsAg seroclearance or HBsAg ≤100 IU/mL at EOFU. Written informed consent were obtained from these patients for the analysis of liver tissue and blood samples. This study was approved by the Institution Review Board, The University of Hong Kong and Hospital Authority Hong Kong West Cluster (UW 18-021).

Analysis of serum HBV markers

Serum HBV DNA was measured by the COBAS HBV Test (Roche Diagnostics GmbH, Mannheim, Germany), with a lower limit of detection (LLOD) of 20 IU/mL. HBsAg was measured by the Elecsys HBsAg Quant II assay (Roche Diagnostics, Indianapolis, IN, USA) with a LLOD of 0.05 IU/mL (the LLOD per the manufacturers’ user manual is 0.04 U/mL in Paul-Ehrlich-Institute standard and 0.1 U/mL in World Health Organization standard). HBcrAg was measured with the Lumipulse HBcrAg assay (FujiRebio Inc., Tokyo, Japan), with a linear range of 1,000 to 10,000,000 U/mL, while the LLOD was 100 U/mL. Values above the LLOD (i.e., 100–1,000 U/mL) are also presented for statistical analysis. This methodology has been verified and used in our previous studies. Circulating HBV RNA was quantified by real-time polymerase chain reaction (PCR) using the Roche HBV RNA investigational assay (IA) for use on the cobas 6800/8800 Systems (Roche Diagnostics, Pleasanton, CA, USA). The HBV RNA assay is a quantitative nucleic acid test (lower limit of quantification 10 copies/mL; linearity range 10 to 10×10 copies/mL on armored RNA template) to enable the detection and quantification of HBV RNA in EDTA plasma or serum of HBV-infected patients. Analytical verification was completed at Roche Development (RKZ) and included genotypes A, B, C, D. Additionally, in-silico analysis indicated the design would perform equivalently on all genotypes. All tests were performed by trained operators in accordance with the manu-
facturers’ specifications.\textsuperscript{36,37} Runs were considered valid if internal controls were valid and no protocol deviations or incidents occurred that might affect the validity of the data. If a run was considered invalid, all samples included in that run were retested wherever possible. HBV genotyping was performed by PCR amplification of the HBV S region, followed by direct sequencing and phylogenetic comparison with reference HBV sequences in the National Center for Biotechnology Information (NCBI) GenBank, as previously described.\textsuperscript{38}

**Analysis of intrahepatic HBV markers**

The extraction of DNA from liver tissues, as well as the data and methods of measurement of intrahepatic total HBV DNA and cccDNA have been reported previously.\textsuperscript{18,33} Briefly, intrahepatic total HBV DNA and cccDNA were isolated by either QIAamp DNA Mini-Kit or the Allprep DNA/RNA/protein (both Qiagen) according to manufacturer’s instructions, and measured by real-time PCR using hybridization probes and primers targeting the S regions and those targeting the nicked region of the HBV DNA genome for intrahepatic total HBV DNA and cccDNA, respectively, with lower limit of quantification of 0.001 and 0.005 copies/cell, respectively.

**Detection of integrated HBV DNA**

Degree of HBV DNA integration were studied in 17 patients with adequate liver tissue samples. Total liver DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to manufacturer’s instructions. DNA quality and quantity were assessed using both the Qubit DNA quantification assay and the NanoDrop™ 2000c spectrophotometers (both from Thermo Fisher Scientific, Waltham, MA, USA).

**Inverse PCR**

HBV DNA integration and the estimation of hepatocyte clone size were measured by inverse PCR.\textsuperscript{39,40} Inverse PCR relies on the selective use of restriction enzymes (NcoI in this case) to cut at both HBV DNA (near the DR1-DR2 region) and the human genome. Following re-ligation of the restricted fragments and further cutting by a second restriction enzyme (BsiHKAI), the fragments were amplified by nested-PCR. In order to detect HBV-human chimeric DNA in small hepatocyte clones, the restricted fragments were serially diluted and PCR-amplified in replicates of 12. The sequence of the primers for the first round PCR were OF1 (nt 1585–1603) 5’-TTGCTTTCACCTCAGCAG-3’ and OR1 (nt 1422–1405) 5’-AAAGACGTTCCCAGCTG-3’, and those for the second round were IF2 (nt 1605–1623) 5’-CGCATGGAGACCACGGTA-3’ and IR2 (nt 1390–1372) -CACAGCTAGCAGCCATG-3’. The identities of the amplicons were confirmed by Sanger sequencing.

**Identification of viral-host junction and hepatocyte clone size calculation**

Sequencing results were aligned with both HBV genome (NCBI accession number: NC_003977.1) and Human Reference Genome (GRCh38) for the identification of viral-host junctions and integration sites.

**Determination of cell number in liver tissues**

The number of cells in the liver tissues were determined by measurement of the human genomic DNA content in the extracted liver DNA using the Light-Cycler Control DNA kit (Roche Molecular Systems), based on an estimation of 6.667 pg human genomic DNA per cell. Intrahepatic total HBV DNA and cccDNA were log transformed and expressed in log copies/liver cell, whereas HBV DNA integration frequency was expressed in integrants/1,000 liver cells.

**Statistical analysis**

Statistical analyses were performed by using IBM SPSS ver. 27.0 (IBM, Armonk, NT, USA) unless otherwise specified. Continuous variables were logarithmic transformed and compared using the Mann-Whitney U test. Related variables were compared by using the Wilcoxon signed rank test. For variables that showed statistical significance at comparison, area under receiver operating curve (AUROC) analysis was performed to assess the overall prediction accuracy. The optimal cut-off values for predicting FHR were derived by maximizing the Youden’s index (sensitivity + specificity – 1) from the AUROC analysis. The time-dependent AUROC was estimated by Inverse Probability of Censoring via package “timeROC” in R (version 4.1.2). To determine whether a biomarker profile was independently associated with FHR, multivariate binary logistic regression was performed using variables that were significant at univariate analysis, with odds ratio (OR) and
95% confidence interval (95% CI) calculated. Time-to-event analysis by Cox regression was performed on the entire cohort after excluding patients with undetectable baseline pgRNA or HBcrAg, with results expressed in hazard ratio (HR) and 95% CI. We were able to determine the time of HBsAg seroclearance from the cohort. For patients who remained HBsAg+ but achieved quantitative HBsAg (qHBsAg) <100 at EOFU, longitudinal qHBsAg data was not available; therefore the time of achieving the ‘event’ was arbitrarily taken as the time of last FU for these low qHBsAg patients. Kaplan-Meier survival analysis was performed to compare the probability of FHR in pre-defined groups, with differences tested for statistical significance by log rank test. A two-tailed \( P < 0.05 \) was considered statistically significant.

### RESULTS

#### Baseline characteristics

A total of 64 patients (71.9% male) were recruited for analysis. At baseline, i.e., NA initiation, the median age of the cohort was 38.2 (interquartile range [IQR], 29.4–47.2) years, with 28 (43.75%) being HBeAg-positive. The median HBcrAg, HBV pgRNA, HBV DNA and HBsAg were 3.54 log U/mL (IQR, 2.3–4.78), 4.00 log copies/mL (IQR, 2.98–4.88), 6.60 log IU/mL (IQR, 5.84–8.24), and 3.48 log U/mL (IQR, 2.89–3.77), respectively. The median total intrahepatic HBV DNA and cccDNA were 140.5 and 3.37 copies/cell, respectively. The frequency of HBV DNA integration per 1,000 liver cells was 0.94 (IQR, 0.51–2.24) (Table 1). Six patients had undetectable serum HBV pgRNA at baseline (HBeAg-positive, 1; HBeAg-negative, 5) and 12 HBeAg-negative patients had undetectable serum

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Value</strong></td>
</tr>
<tr>
<td>Gender (% male)</td>
</tr>
<tr>
<td>Age at recruitment (years)</td>
</tr>
<tr>
<td>Antiviral therapy</td>
</tr>
<tr>
<td>Adefovir</td>
</tr>
<tr>
<td>Clevudine</td>
</tr>
<tr>
<td>Entecavir</td>
</tr>
<tr>
<td>Lamivudine</td>
</tr>
<tr>
<td>Telbivudine</td>
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<tr>
<td>HBeAg (% positive)</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>ALT (U/L)</td>
</tr>
<tr>
<td>HBcrAg (log U/mL)</td>
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<tr>
<td>HBV pgRNA (log copies/mL)</td>
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<tr>
<td>HBV DNA (log IU/mL)</td>
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<tr>
<td>HBsAg (log IU/mL)</td>
</tr>
<tr>
<td>Intrahepatic total HBV DNA (log copies/liver cell)</td>
</tr>
<tr>
<td>Intrahepatic cccDNA (log copies/ liver cell)</td>
</tr>
<tr>
<td>HBV DNA integration frequency (integrants/1,000 liver cells)*</td>
</tr>
</tbody>
</table>

Values are presented as median (% or median) (interquartile range).

HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA.

*Data available in 17 patients.
HBcrAg at baseline. Among these 12 patients with undetectable serum HBcrAg, four also had undetectable serum HBV pgRNA (Supplementary Table 1).

The median duration from start of NA to EOFU was 17.1 (IQR, 12.8–18.2) years. At EOFU, 22/64 patients (34.4%) achieved FHR, including eight patients with HBsAg seroclearance (median duration from NA, 11.9 years [IQR, 3.97–15.96]), and 14 patients with HBsAg ≤100 IU/mL. No significant differences in achieving FHR were observed between ETV-treated and non-ETV-treated patients (43.8% vs. 31.3%, P=0.269). Since the levels of HBV biomarkers are highly dependent on HBeAg status,19,34,41 analyses were performed in HBeAg-positive and HBeAg-negative patients separately.

HBeAg-positive patients

Kinetics of viral markers during initial 48 weeks of NA therapy

At baseline, median serum HBV pgRNA was 3 logs lower than HBV DNA level (4.92 log copies/mL vs. 8.13 log IU/mL, respectively). After 4 weeks of NA, both markers were reduced but a bigger decline was observed for HBV DNA compared to HBV pgRNA, leading to narrowing of the gap between the two markers (4.10 log copies/mL for HBV pgRNA vs. 4.33 log IU/mL for HBV DNA). At week 12, HBV pgRNA caught up with the level of HBV DNA (3.01 log copies/mL vs. 3.07 log IU/mL, respectively) and after which the relationship reversed that serum pgRNA levels were higher than HBV DNA levels at week 24 (2.61 log copies/mL vs. 2.22 log IU/mL, respectively) and week 36 (2.54 log copies/mL vs. 1.83 log IU/mL, respectively). At week 48, the median serum levels of both markers were undetectable. Overall, there was a gradual reduction of median serum HBcrAg level except with two blips at week 12 and week 36. At week 48, there was a 5.15 log IU/mL reduction of serum HBcrAg from baseline, and was still detectable at a median level of 3.04 log U/mL (Fig. 1A, Supplementary Table 2).

Kinetics of viral markers in FHR vs. non-FHR

Among 28 HBeAg-positive patients, 26 patients (92.9%) achieved HBeAg seroclearance as of EOFU. Six of the 28 patients (21.4%) achieved FHR at EOFU (three achieved HBsAg seroclearance and the other three had HBsAg level ≤100 IU/mL). There were no statistical differences in the baseline demographics including age and gender, the duration of follow-up as well as the median levels of HBcrAg, HBV DNA and HBsAg. Notably, patients with FHR had a higher baseline median HBV pgRNA level compared with patients without FHR. All these parameters at baseline and during the initial 48 weeks of NA therapy are shown in Supplementary Table 3. Supplementary Figure 1A, B shows the levels of HBcrAg and HBV pgRNA at each time point.

Figure 1. Median levels of HBV pgRNA, HBV DNA and HBcrAg during initial 48 weeks of nucleos(t)ide analogues therapy. Lower limit of quantification for HBV pgRNA = 10 copies/mL (i.e., 1 log_{10} copies/mL); lower limit of detection for HBV DNA = 20 IU/mL (i.e., 3 log_{10} IU/mL); lower limit of detection for HBcrAg = 100 U/mL (i.e., 2 log_{10} U/mL). (A) HBeAg-positive patients. (B) HBeAg-negative patients. HBeAg, hepatitis B e antigen; pgRNA, pre-genomic RNA; HBV, hepatitis B virus; HBcrAg, hepatitis B core-related antigen.
Association between early on-treatment changes of serum viral markers and FHR

One patient with baseline undetectable serum HBV pgRNA was excluded from the individual biomarker analysis. Compared to non-FHR, FHR patients had higher median decline in most serum HBV markers including HBcrAg, pgRNA and HBV DNA at all the time points measured during the first year of NA treatment (Table 2). These differences reached statistically significant levels at all time points except for HBV DNA at week 4.

Table 2. Median reduction in serum and intrahepatic viral markers at different time points during initial 48 weeks of NA therapy among initially HBeAg-positive patients

<table>
<thead>
<tr>
<th>Viral marker</th>
<th>FHR</th>
<th>Non-FHR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBcrAg (log U/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>4.88 (-3.15 to 5.85)</td>
<td>0.77 (-4.08 to 5.39)</td>
<td>0.395</td>
</tr>
<tr>
<td>Week 12</td>
<td>5.30 (2.26 to 5.95)</td>
<td>4.33 (3.54 to 5.43)</td>
<td>0.141</td>
</tr>
<tr>
<td>Week 24</td>
<td>5.22 (1.77 to 6.02)</td>
<td>4.32 (3.33 to 5.49)</td>
<td>0.387</td>
</tr>
<tr>
<td>Week 36</td>
<td>5.68 (4.49 to 6.36)</td>
<td>4.59 (3.68 to 5.40)</td>
<td>0.199</td>
</tr>
<tr>
<td>Week 48</td>
<td>5.50 (4.63 to 6.03)</td>
<td>4.90 (4.28 to 5.60)</td>
<td>0.283</td>
</tr>
<tr>
<td>HBV pgRNA (log copies/mL)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>5.49 (5.19 to 5.83)†</td>
<td>4.32 (3.30 to 5.18)†</td>
<td>0.016†</td>
</tr>
<tr>
<td>Week 12</td>
<td>5.58 (5.06 to 6.05)†</td>
<td>4.38 (3.76 to 5.15)†</td>
<td>0.037†</td>
</tr>
<tr>
<td>Week 24</td>
<td>5.63 (5.22 to 5.96)†</td>
<td>4.40 (3.65 to 5.45)†</td>
<td>0.047†</td>
</tr>
<tr>
<td>Week 36</td>
<td>5.68 (4.50 to 6.36)†</td>
<td>4.59 (3.68 to 5.40)†</td>
<td>0.030†</td>
</tr>
<tr>
<td>Week 48</td>
<td>5.63 (5.21 to 5.99)†</td>
<td>4.42 (4.42 to 5.24)†</td>
<td>0.016†</td>
</tr>
<tr>
<td>HBV DNA (log IU/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>8.26 (7.63 to 8.78)</td>
<td>7.82 (6.29 to 8.55)</td>
<td>0.427</td>
</tr>
<tr>
<td>Week 12</td>
<td>8.26 (7.63 to 8.78)</td>
<td>7.82 (6.29 to 8.55)</td>
<td>0.427</td>
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<tr>
<td>Week 24</td>
<td>8.26 (7.63 to 8.78)</td>
<td>7.82 (6.29 to 8.55)</td>
<td>0.387</td>
</tr>
<tr>
<td>Week 36</td>
<td>8.30 (7.23 to 8.93)</td>
<td>7.59 (6.22 to 8.46)</td>
<td>0.336</td>
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<tr>
<td>Week 48</td>
<td>8.26 (7.63 to 8.78)</td>
<td>7.82 (6.29 to 8.55)</td>
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<td>HBsAg (log IU/mL)</td>
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<tr>
<td>Week 4</td>
<td>0.03 (-4.05 to 4.80)</td>
<td>3.13 (1.90 to 3.42)</td>
<td>0.642</td>
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<tr>
<td>Week 12</td>
<td>-3.63 (-4.20 to 4.83)</td>
<td>1.53 (-3.5 to 3.62)</td>
<td>0.581</td>
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<tr>
<td>Week 24</td>
<td>1.17 (-3.13 to 4.95)</td>
<td>3.34 (2.16 to 4.12)</td>
<td>0.682</td>
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<tr>
<td>Week 36</td>
<td>3.31 (-0.41 to 5.03)</td>
<td>3.06 (-2.89 to 3.78)</td>
<td>0.538</td>
</tr>
<tr>
<td>Week 48</td>
<td>3.65 (0.82 to 5.02)</td>
<td>2.75 (-3.11 to 3.62)</td>
<td>0.336</td>
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<tr>
<td>Intrahepatic total HBV DNA (log copies/liver cell)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 48</td>
<td>2.42 (2.16 to 3.07)</td>
<td>2.74 (2.01 to 3.13)</td>
<td>0.764</td>
</tr>
<tr>
<td>Intrahepatic cccDNA (log copies/liver cell)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Week 48</td>
<td>0.65 (0.27 to 1.71)</td>
<td>1.06 (0.74 to 1.38)</td>
<td>0.494</td>
</tr>
<tr>
<td>HBV DNA integration frequency (integrants/1,000 liver cells)‡</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Week 48</td>
<td>1.56 (0.30 to not applicable)§</td>
<td>0.07 (-0.91 to 0.65)</td>
<td>0.381</td>
</tr>
</tbody>
</table>

NA, nucleos(t)ide analogues; HBeAg, hepatitis B e antigen; FHR, favourable HBsAg response; HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; pgRNA, pre-genomic RNA; HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA.

*Excluded 1 patient with undetectable baseline HBV pgRNA.

†Significant variables.

‡Data available in 7 patients.

§Data available in only two patients in the FHR group.
cally significant level at all treatment time points throughout for pgRNA. Importantly, pgRNA decline was significantly greater in FHR patients compared to non-FHR patients starting as early as week 4 (5.49 vs. 4.32 log copies/mL decline, \(P=0.016\)) (Table 2, Fig. 2A) despite a higher median HBV pgRNA level at baseline (5.63 vs. 4.43 log copies/mL, \(P=0.024\)) (Supplementary Table 3).

ROC analysis was performed to assess the performance characteristics of decline in serum HBV pgRNA at various time points to predict FHR at EOFU. The AUROC values were highest and identical for week 4 and week 48 decline, both being 0.825 (95% CI, 0.661–0.989, \(P<0.001\)) (Supplementary Table 4). For week 4 decline in serum pgRNA, adopting the Youden’s index to identify the cut-off level of \(\geq 5.32\) log copies/mL reduction, the sensitivity and specificity for FHR at EOFU were 83.3% and 85%, respectively. Using the cut-off of \(\geq 5.32\) log copies/mL drop at week 4, 62.5% patients developed FHR compared to 5.6% remaining patients \(P=0.004\).

For week 48 decline in serum pgRNA, using a cut-off level of \(>5.31\) log copies/mL reduction, the sensitivity and specificity for FHR at EOFU were 83.3% and 85%, respectively. Using the cut-off of \(\geq 5.31\) log copies/mL drop at week 48, 62.5% patients developed FHR compared to 5.6% remaining patients \(P=0.004\). Time-dependent AUROC was performed on for week 4, 12, 24, 36, and 48 drop of HBV pgRNA and the results are shown in Supplementary Table 5.

**Intrahepatic viral markers and FHR**

The levels of intrahepatic total HBV DNA, cccDNA and the frequency of HBV DNA integration at baseline were not significantly different between FHR and non-FHR patients (Supplementary Table 3). For all HBeAg-positive patients, upon 48 weeks of NA therapy, the median level of cccDNA reduced from 1.096 to 0.005 log copies per cell \(P<0.001\), while that for intrahepatic total HBV DNA reduced from 2.648 to 0.554 log copies per cell \(P<0.001\). The frequency of HBV integration decreased from 3.96 (IQR, 0.82–NA) at week 0 to 2.40 (0.52–NA) integrants/1,000 liver cells at week 48 for FHR group \(P=0.18\), compared to 1.48 (IQR, 0.82–2.00) to 1.10 (0.52–2.49) integrants/1,000 liver cells for non-FHR group \(P=0.31\). No significant differences in the degree of decline in intrahepatic viral markers or HBV DNA integration frequency were observed between FHR and non-FHR patients (Table 2).

**HBeAg-negative patients**

**Kinetics of viral markers during initial 48 weeks of NA therapy**

At baseline, median serum HBV pgRNA was 3 log lower than HBV DNA level (3.34 log copies/mL vs. 6.20 log IU/mL,
respectively). At week 12, the median serum levels of HBV pgRNA, HBV DNA and HBcrAg were undetectable (Fig. 1B, Supplementary Table 2).

**Kinetics of viral markers in FHR vs. non-FHR**

Among 36 HBeAg-negative patients, 16 patients (44.4%) achieved FHR at EOFU (five achieved HBsAg seroclearance and the other 11 had HBsAg level ≤100 IU/mL). The baseline

| Table 3. Median reduction in serum and intrahepatic viral markers at different time points during initial 48 weeks of NA therapy among initially HBeAg-negative patients |
|-----------------|-----------------|-----------------|-----------------|
| **Viral marker** | **FHR**         | **Non-FHR**     | **P-value**     |
| **HBcrAg (log U/mL)** |                 |                 |                 |
| Week 4           | 2.98 (2.20 to 3.55) | -1.75 (-2.65 to 2.53) | 0.023†         |
| Week 12          | 3.21 (2.68 to 3.56) | 2.38 (-0.07 to 3.39) | 0.120          |
| Week 24          | 3.25 (2.24 to 3.56) | 2.94 (1.64 to 3.43) | 0.376          |
| Week 36          | 3.17 (2.40 to 3.56) | 2.94 (1.74 to 3.43) | 0.413          |
| Week 48          | 3.20 (2.63 to 3.58) | 2.95 (1.93 to 3.40) | 0.264          |
| **HBV pgRNA (log copies/mL)** |                 |                 |                 |
| Week 4           | 3.93 (3.01 to 4.01) | 3.21 (2.42 to 4.40) | 0.594          |
| Week 12          | 3.93 (3.03 to 4.02) | 3.47 (2.41 to 4.42) | 1.000          |
| Week 24          | 3.95 (3.02 to 4.03) | 3.53 (2.54 to 4.51) | 0.692          |
| Week 36          | 3.93 (3.01 to 4.02) | 3.55 (2.58 to 4.53) | 0.708          |
| Week 48          | 3.93 (3.00 to 4.02) | 3.55 (2.58 to 4.52) | 0.679          |
| **HBV DNA (log IU/mL)** |                 |                 |                 |
| Week 4           | 5.86 (5.30 to 6.37) | 6.43 (5.58 to 7.79) | 0.140          |
| Week 12          | 5.86 (5.30 to 6.38) | 6.32 (5.29 to 7.79) | 0.320          |
| Week 24          | 5.85 (5.23 to 6.32) | 6.43 (5.59 to 7.79) | 0.064          |
| Week 36          | 5.85 (5.23 to 6.4)  | 6.43 (5.59 to 7.79) | 0.169          |
| Week 48          | 5.86 (5.3 to 6.38)  | 6.43 (5.59 to 7.79) | 0.149          |
| **HBsAg (log IU/mL)** |                 |                 |                 |
| Week 4           | 2.17 (-1.06 to 3.27) | 2.82 (-0.44 to 3.37) | 0.290          |
| Week 12          | 2.52 (-0.80 to 3.06) | 2.86 (-0.78 to 3.19) | 0.422          |
| Week 24          | 2.14 (-2.66 to 3.21) | -2.80 (-3.45 to 3.03) | 0.189          |
| Week 36          | 2.68 (-1.19 to 3.16) | -2.15 (-3.17 to 3.08) | 0.236          |
| Week 48          | -1.89 (-3.03 to 2.70) | -2.92 (-3.47 to 2.76) | 0.386          |
| **Intrahepatic total HBV DNA (log copies/liver cell)** |                 |                 |                 |
| Week 48          | 1.42 (0.21 to 2.23) | 1.85 (1.21 to 2.40) | 0.236          |
| **Intrahepatic cccDNA (log copies/liver cell)** |                 |                 |                 |
| Week 48          | 0.45 (1.04 to 0.39) | 0.04 (-0.35 to 0.32) | 0.479          |
| **HBV DNA integration frequency (integrants/1,000 liver cells)** |                 |                 |                 |
| Week 48          | 0.11 (-0.13 to 0.47) | 0.30 (-3.01 to 3.57) | 0.762          |

NA, nucleos(t)ide analogues; HBeAg, hepatitis B e antigen; FHR, favourable HBsAg response; HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; pgRNA, pre-genomic RNA; HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA.

*Excluded 12 patients with undetectable baseline HBcrAg.
†Significant variables.
‡Excluded 5 patients with undetectable baseline HBV pgRNA.
§Data available in 10 patients.
characteristics of patients and the median levels of HBcrAg, HBV pgRNA, HBV DNA and HBsAg during the initial 48 weeks of NA therapy were compared between FHR and non-FHR patients and shown in Supplementary Table 6. Patients who achieved FHR were significantly younger at baseline (41.5 vs. 46.4 years old, \( P = 0.026 \)) and had longer duration of follow-up (18.1 vs. 13.1 years, \( P = 0.033 \)) compared to non-FHR patients (Supplementary Table 6). Supplementary Figure 1C, D shows the levels of HBcrAg and HBV pgRNA at each time point.

**Association between early on-treatment changes of serum viral markers and FHR**

Patients with baseline undetectable serum viral biomarker (5/36 for HBV pgRNA and 12/36 for HBcrAg) were excluded from the individual biomarker analysis. Compared to non-FHR patients, FHR patients had median reduction in HBcrAg at week 4 (increment of 1.75 vs. reduction of 2.98 log U/mL; \( P = 0.023 \)) (Fig. 2B). There were no significant differences in the median log reduction for HBV pgRNA, HBV DNA and HBsAg (Table 3).

ROC analysis was performed to assess the performance characteristics of week 4 change in serum HBcrAg to predict FHR at EOFU. The AUROC is 0.789 (95% CI, 0.596–0.982; \( P = 0.003 \)). Using a cut-off level of \( \geq 2.05 \) log U/mL decline, the sensitivity and specificity for FHR at EOFU were 75% and 62.5%, respectively. Using the cut-off of \( \geq 2.05 \) log U/mL decline in HBcrAg at week 4, 63.6% patients developed FHR compared to 7.7% remaining patients. Binary logistic regression inputting the factors of age, duration of follow-up and week 4 HBcrAg decline showed that only week 4 HBcrAg decline of \( \geq 2.05 \) log U/mL remained significantly associated with FHR at EOFU (OR, 16.919; 95% CI, 11.245–229.964; \( P = 0.034 \)).

**Intrahepatic viral markers and FHR**

The levels of intrahepatic total HBV DNA, cccDNA and the frequency of HBV DNA integration at baseline were not significantly different between FHR and non-FHR patients (Supplementary Table 6). For all HBeAg-negative patients, upon 48 weeks of NA therapy, the median level of cccDNA reduced from 0.041 to -0.832 log copies per cell (\( P < 0.001 \)), while that for intrahepatic total HBV DNA reduced from 1.792 to 0.114 log copies per cell (\( P < 0.001 \)). The frequency of HBV integration was 0.71 (IQR, 0.41–1.86) at week 0 and 0.70 (0.34–1.50) integrants/1,000 liver cells at week 48 for FHR group; \( P = 0.46 \), whereas for non-FHR group the integration frequency non-significantly increased from 1.60 (IQR, 0.32–4.48) to 2.15 (0.08–4.10) integrants/1,000 liver cells; \( P = 0.60 \). No significant differences in the degree of decline in both intrahepatic viral markers or the frequency of HBV DNA integration were observed between FHR and non-FHR patients (Table 3).

**Serum HBV markers and cccDNA reduction**

We analysed the decline of various serum HBV biomarkers with respect to cccDNA reduction at week 48. As it was previously reported that the magnitude of cccDNA decline after 1 year of antiviral therapy was about 1 log copy/cell, \(^{42}\) we performed subgroup analysis for patients with \( \geq 1 \) log copy/cell reduction (n=17) vs. \(< 1 \) log copy/cell reduction (n=47) of cccDNA at 48 weeks of NA. Compared to patients with \(< 1 \) log decline of cccDNA, patients with \( \geq 1 \) log decline of cccDNA had significantly bigger reductions in serum HBV RNA (week 12, 24, and 48), HBcrAg (week 12, 24, 36, and 48) and HBV DNA (week 4, 12, 24, 36, and 48) (Table 4).

**Composite endpoint and time-to-event analysis**

A composite variable ‘week 4 biomarker response’ consisting of either week 4 pgRNA decline \( \geq 5.32 \) log copies/mL for HBeAg+ or week 4 HBcrAg decline \( \geq 2.05 \) log U/mL for HBeAg− was computed into the analysis. The result showed that ‘week 4 biomarker response’ was significantly associated with FHR (HR, 10.378; 95% CI, 2.312–46.589; \( P = 0.002 \)) (Fig. 3).

**DISCUSSION**

Functional cure is a desirable endpoint yet a rare occurrence in CHB patients on long-term NA therapy. The ‘stop-to-cure’ approach has been adopted to induce host immunity triggered by recurrence of HBV replication. The probability of NA cessation-induced functional cure is increased if cccDNA transcriptional activity has been silenced to some degree during treatment. \(^{43}\) The end-of-treatment viral biomarkers including HBsAg and HBV pgRNA have been reported to predict post NA-cessation virological flare. \(^{44,45}\) Importantly, the end-of-treatment HBsAg titre is a crucial factor that determines the fate after antiviral therapy cessation.
In this study, we studied the early on-treatment viral markers in a group of CHB patients who were started on NA with long-term follow-up. We found that among those who achieved HBsAg seroclearance or low HBsAg (≤100 IU/mL) after on NA > 10 years, the early on-treatment changes in serum viral biomarkers were significantly different compared to those who did not achieve a low HBsAg level. Specifically, for HBeAg-positive patients, all viral biomarkers (except HBsAg) showed the same trend (albeit statistically insignificant for HBV DNA and HBcrAg) that a bigger decline in the individual biomarkers were observed in FHR patients compared to non-FHR. Importantly, serum HBV pgRNA was the most useful in detecting early on-treatment differences—a high statistical power was observed despite a limited number of patients were included. For HBeAg-negative patients, both serum HBcrAg and HBV pgRNA showed similar pattern that a bigger decline in the individual biomarkers were observed in FHR patients compared to non-FHR. Due to the fact that a sizable proportion of HBeAg-negative patients had undetectable HBcrAg (12/36, 33.3%) or HBV pgRNA (5/36, 13.9%) at baseline, the statistical power of these serum biomarkers was further reduced. Nevertheless, this is the first study to describe the role of early on-treatment viral markers in prediction of favourable HBsAg suppression in NA-treated CHB patients.

In HBeAg-negative patients, we noted that those who

Table 4. Differential reductions of serum viral markers at week 48 with respect to cccDNA decline

<table>
<thead>
<tr>
<th>cccDNA ≥1 log copy/cell reduction (n=17)</th>
<th>cccDNA &lt;1 log copy/cell reduction (n=47)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA log decline*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>4.39 (3.26 to 5.47)</td>
<td>3.93 (3.00 to 4.66)</td>
</tr>
<tr>
<td>Week 12</td>
<td>4.38 (3.98 to 5.43)</td>
<td>3.97 (1.02 to 4.63)</td>
</tr>
<tr>
<td>Week 24</td>
<td>4.43 (3.99 to 5.65)</td>
<td>3.97 (3.04 to 4.58)</td>
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<tr>
<td>Week 36</td>
<td>4.39 (3.92 to 5.44)</td>
<td>3.97 (3.05 to 4.55)</td>
</tr>
<tr>
<td>Week 48</td>
<td>4.43 (4.00 to 5.61)</td>
<td>3.97 (3.04 to 4.62)</td>
</tr>
<tr>
<td>HBcrAg log decline†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>3.73 (-4.04 to 5.41)</td>
<td>-0.88 (-2.65 to 3.49)</td>
</tr>
<tr>
<td>Week 12</td>
<td>4.36 (3.93 to 5.44)</td>
<td>3.02 (1.68 to 4.14)</td>
</tr>
<tr>
<td>Week 24</td>
<td>4.36 (3.84 to 5.60)</td>
<td>3.18 (1.79 to 4.12)</td>
</tr>
<tr>
<td>Week 36</td>
<td>4.40 (3.59 to 5.60)</td>
<td>3.25 (2.39 to 4.39)</td>
</tr>
<tr>
<td>Week 48</td>
<td>4.80 (4.25 to 5.65)</td>
<td>3.40 (2.50 to 4.50)</td>
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<tr>
<td>HBV DNA log decline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>8.22 (7.50 to 8.59)</td>
<td>6.32 (5.51 to 7.87)</td>
</tr>
<tr>
<td>Week 12</td>
<td>8.22 (7.50 to 8.59)</td>
<td>6.30 (5.51 to 7.87)</td>
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<tr>
<td>Week 24</td>
<td>8.22 (7.50 to 8.59)</td>
<td>6.31 (5.51 to 7.59)</td>
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<tr>
<td>Week 36</td>
<td>8.04 (7.42 to 8.59)</td>
<td>6.32 (5.52 to 7.73)</td>
</tr>
<tr>
<td>Week 48</td>
<td>8.22 (7.50 to 8.59)</td>
<td>6.32 (5.52 to 7.87)</td>
</tr>
<tr>
<td>HBsAg log decline</td>
<td></td>
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</tr>
<tr>
<td>Week 4</td>
<td>2.97 (-3.06 to 3.42)</td>
<td>2.88 (1.39 to 3.39)</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.75 (-3.57 to 3.45)</td>
<td>2.50 (-2.24 to 3.30)</td>
</tr>
<tr>
<td>Week 24</td>
<td>3.10 (-1.84 to 4.09)</td>
<td>-2.12 (-3.15 to 3.44)</td>
</tr>
<tr>
<td>Week 36</td>
<td>3.06 (1.33 to 3.78)</td>
<td>2.14 (-2.87 to 3.24)</td>
</tr>
<tr>
<td>Week 48</td>
<td>2.75 (-3.12 to 3.42)</td>
<td>-2.66 (-3.15 to 3.02)</td>
</tr>
</tbody>
</table>

*Excluded 5 patients with baseline undetectable HBV RNA.
†Excluded 12 patients with baseline undetectable HBcrAg.

cccDNA, covalently closed circular DNA; HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

*Excluded 5 patients with baseline undetectable HBV RNA.
†Excluded 12 patients with baseline undetectable HBcrAg.
achieved FHR were significantly younger at baseline and had longer duration of follow-up compared to patients who did not achieve FHR. It is unknown whether initiating NA at an earlier age is associated with higher chance of HBsAg seroclearance. Also, extension of follow-up duration would potentially identify more cases with HBsAg seroclearance. We additionally performed multivariate analysis which showed that the week 4 decline in HBcrAg was the only significant factor associated with FHR among HBeAg-negative patients (Supplementary Table 7).

Among the four serum biomarkers, HBsAg levels were the least suppressed by NA (Table 2, Supplementary Table 2). Because HBsAg is not a direct target of NAs and that it can be expressed from integrated HBV DNA, the decline of HBsAg by NAs is often very small. Therefore, HBsAg remained relatively static (3–4 logs for HBeAg-positive and 2–3 logs for HBeAg-negative patients) during the initial 48 weeks of NA therapy in contrast to the substantial reduction in serum HBV DNA (direct target of NA), HBcrAg and HBV pgRNA (both are surrogate markers of cccDNA transcriptional activity). Also, while the differential reductions of other three markers (HBV pgRNA, HBcrAg and HBV DNA) were reflective of whether cccDNA was suppressed effectively, HBsAg levels did not show similar trends (Table 4). Therefore, early on-treatment HBsAg kinetics could not predict subsequent levels upon long-term NA nor the degree of cccDNA suppression.

Intrahepatic viral markers were significantly suppressed upon initial 48 weeks of NA therapy and was consistent with other reports in the literature. In contrast to serum biomarkers, the dynamics of intrahepatic total HBV DNA or cccDNA during initial 48 weeks of NA were not predictive of FHR. It was previously reported that most patients develop virological rebound despite undetectable cccDNA after >10 years of NA. It is tempting to speculate that what matters most is the degree of cccDNA silencing, instead of the absolute amount of viral template that is still present, that determines the ultimate fate of whether NA can be used in a finite manner. In turn, the degree of cccDNA silencing can be re-
flected by the magnitude of reduction of serum viral biomarkers, namely HBcrAg or HBV pgRNA, during the course of NA. The fact that the rapid reduction of these biomarkers as early as week 4 might indicate a high degree of cccDNA silencing which was clinically translated into a long term favourable HBsAg response to NA (Fig. 4). Achieving HBsAg ≤100 IU/mL is the pre-requisite for consideration of stopping NA and is expected to have favourable rate of subsequent HBsAg seroclearance. However, our study cannot address whether early biomarker response can help to predict successful off-NA virological control. We propose that for subjects without week 4 biomarker response—indicating a low chance of achieving functional cure or a low HBsAg titre by long-term NA alone—NA should be continued and they should be prioritized for clinical trials with novel anti-HBV therapies within the HBV functional cure program (Fig. 4). Currently, many trials that are designed for NA-treated CHB patients would like to consider qHBsAg and/or HBV DNA as the inclusion criteria, while the role of other biomarkers is seldom explored. Early on-NA biomarker response will help to identify patients potentially eligible for clinical trials who would otherwise be embarked on indefinite duration of NA therapy. Of note, a high frequency of HBV DNA integration events at baseline was observed in both HBeAg-positive and HBeAg-negative patients. FHR was found to be independent of the degree of HBV DNA integration at baseline. Our group previously reported that HBV DNA integration frequency significantly decreased after long-term NA >10 years. It will be worth investigating whether the reduction in HBV DNA integration is associated with FHR after long-term NA therapy.

In the six patients with baseline undetectable HBV pgRNA, four had undetectable HBcrAg, while the HBcrAg levels were 3.07 and 5.63 log U/mL for the remaining two patients. The serum HBV DNA was 2.82, 5.23, 5.85, 6.46, 7.99, and 8.32 log IU/mL for these six patients (Supplementary Table 1). This RNA-DNA dissociation was mainly observed in HBeAg-negative patients. The use of serum viral biomarkers especially HBV pgRNA to predict FHR would therefore be more useful

\[\text{Figure 4. Schematic diagram illustrating the relationship between NA-induced cccDNA silencing and long-term effects on HBsAg suppression. During early phase of NA therapy, HBsAg levels remain relatively static despite marked reduction in transcriptional activity as reflected by early biomarker response (i.e., week 4 HBV pgRNA and HBcrAg). The long-term effects of NA on HBsAg production can potentially be predicted using early changes in serum viral biomarkers. HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; pgRNA, pre-genomic RNA; HBcAg, hepatitis B core-related antigen. \#Week 4 biomarker response: a composite variable consisting of either one of the following: 1) week 4 pgRNA decline ≥5.32 log copies/mL (for HBeAg+) and 2) week 4 HBcrAg decline ≥2.05 log U/mL (for HBeAg).}\]
Lung-Yi Mak, et al. 
Early changes in biomarkers predict HBsAg response
http://www.e-cmh.org https://doi.org/10.3350/cmh.2022.0172

in HBeAg-positive patients. Assays with higher sensitivity, i.e., lower limits of detection are likely helpful to quantify the serum viral biomarkers in HBeAg-negative patients. There are a few potential reasons for the observed difference in roles for HBV pgRNA and HBcrAg in predicting FHR in HBeAg-positive and HBeAg-negative patients, respectively. Firstly, because HBeAg is part of the HBcrAg protein, HBeAg expression might have outnumbered HBcrAg expression in the blood for HBeAg-positive patients. Secondly, although both HBV pgRNA and HBcrAg come from cccDNA, their mechanisms of production are slightly different. The production of HBV pgRNA reflects the transcriptional activity, while the production of HBcrAg is affected by both transcription and post-transcriptional (e.g., translational) regulation. Although it is just a speculation, it is possible that different translation efficiencies of HBcrAg exist between HBeAg-positive and HBeAg-negative patients. Thirdly, the small sample size of this study might have masked any true predictive power of either biomarker in patients with different HBeAg status.

The present study has a few limitations. First, we did not have serial blood stored after 48 weeks of NA to study the longitudinal changes of viral biomarkers. Data from previous reports (NA up to 5 years) showed that HBcrAg and HBV pgRNA will continue to decline with increasing duration of therapy. \(^{19,45}\) Second, the limit in the amount of available liver tissues precluded the measurement of intrahepatic HBV pgRNA levels, which would have provided a more comprehensive picture of the impact of NAs on intrahepatic HBV replication. Third, this study is limited to only patients with HBV genotypes B and C and is limited by a small number of patients with available serum and liver biopsies. Besides, the statistical comparisons were limited to non-parametric tests between FHR and non-FHR groups due to multi-collinearity of the biomarkers at different time points. Theoretically, a one-off measurement (instead of multiple timepoints) of biomarker in the early phase of NA might be sufficient to compare the change from baseline and decide if a patient would likely achieve FHR or not in the long run. Therefore, these findings need to be validated by future studies with larger sample size. Lastly, the present study employed only an IA for serum HBV pgRNA measurement, as a standardized serum HBV pgRNA assay is not available at present. Although there is lack of data concerning the stability of serum samples for HBV pgRNA analysis upon long-term storage, previous studies have demonstrated feasibility of this approach,\(^{15,36}\) which is further supported by the overall pattern of pgRNA mirroring that of serum HBV DNA in the current study (Fig. 1). Future studies addressing these technical issues would be helpful to improve utilization of pgRNA assays.

In conclusion, this study demonstrated that early on-treatment viral biomarkers (HBV pgRNA or HBcrAg) can potentially predict low/undetectable HBsAg after long-term NA therapy. Patients without early biomarker response likely need indefinite duration of NA therapy, therefore should be prioritized for enrolment into clinical trials.

**Authors’ contribution**

LYM and DW were responsible for data collection, data analysis and drafting of the manuscript. AK, MH, AH, NC and XM were responsible for data analysis, data interpretation, and critical revision of the article. WKS and MFY were responsible for conception of the work and critical approval of the article.

**Acknowledgements**

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**Conflicts of Interest**

D Wong received speaker’s fees from Abbott Laboratories. A Kuchta and A Hamilton are employees of Roche Molecular Diagnostics. M Hilfiker is an employee of Roche Diagnostics Int. AG. WK Seto received speaker’s fees from AstraZeneca and Mylan, is an advisory board member of CSL Behring, is an advisory board member and received speaker’s fees from AbbVie, and is an advisory board member, received speaker’s fees and researching funding from Gilead Sciences. MF Yuen serves as advisor/consultant for AbbVie, Assembly Biosciences, Aligos Therapeutics, Arbutus Biopharma, Bristol Myer Squibb, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, Hoffmann-La Roche and Springbank Pharmaceuticals, Vir Biotechnology and receives grant/research support from Assembly Biosciences, Aligos Therapeutics, Arrowhead Pharmaceuticals, Bristol Myer Squibb, Fujirebio Incorporation, Gilead Sciences,
Immunocore, Merck Sharp and Dohme, Hoffmann-La Roche, Springbank Pharmaceuticals and Sysmex Corporation. The remaining authors have no conflict of interests.

SUPPLEMENTARY MATERIAL

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

REFERENCES


Letter to the Editor

Letter regarding “Auranofin attenuates hepatic steatosis and fibrosis in nonalcoholic fatty liver disease via NRF2 and NF-κB signaling pathways”

Yuanbin Liu and Mingkai Chen
Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan, China

Keywords: Non-alcoholic fatty liver disease; Auranofin

Dear Editor,

We read with great interest the recently published article by Lee and colleagues,1 which demonstrated that auranofin, a gold compound, can inhibit the progression of nonalcoholic steatohepatitis (NASH) in both in vivo and in vitro models. This study revealed that auranofin reduced fibrosis and the expression of nuclear factor kappa B (NF-κB) and inhibitor of NF-κB alpha in LX-2 cells; while in HepG2 cells, auranofin increased nuclear erythroid 2-related factor 2 expression and significantly reduced inflammation and adipogenesis.1 Furthermore, auranofin has also been shown to impede disease progression in fibrosis and NASH models.1 This study is of great importance as there is currently no effective pharmacological treatment for nonalcoholic fatty liver disease (NAFLD), and auranofin may have potential for repurposing in NAFLD as an agent historically used in rheumatoid arthritis. However, as a reintroduced compound that may have beneficial effects in NAFLD, we would like to provide additional insights regarding the role of auranofin in NAFLD.

Hepatic inflammatory infiltration is a significant feature in NAFLD. Hwangbo et al.2 suggested that auranofin could reduce the expression of inflammatory markers, including the NOD-like receptor family pyrin domain containing 3, in NAFLD and inhibit hepatic steatosis in both in vivo and in vitro models. These results confirmed that auranofin also has anti-inflammatory properties in NAFLD. A recent study combining in silico screen, in vivo and in vitro models demonstrated the antidiabetic effects of auranofin at the 1 mg/kg dose in obese mice fed with high-fat diet.3 Auranofin was proved to accumulate in the white adipose tissue of obese mice, improve insulin sensitivity, exert anti-inflammatory effects, and abolish fatty liver disease.3 Notably, auranofin reduced serum leptin levels, and intact leptin signaling was required for auranofin to exhibit insulin sensitizing effects.3 The presence of hyperleptinemia and leptin resistance in obese patients suggest that partial reduction of leptin by auranofin may be used as a strategy against obesity and NAFLD. The interferon regulatory factor 3 (IRF3) signaling pathway promotes hepatocyte inflammation and apoptosis in NAFLD.4 Auranofin inhibited fatty acid-induced hepatocyte apoptosis in an in vitro model by inducing cellular autophagy and thereby degrading IRF3.5 Auranofin is also known as a pan-inhibitor of thioredoxin

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reductase (TrxR). By inhibiting TrxR, auranofin induced apoptosis in hepatocellular carcinoma (HCC) cells and inhibited tumor growth, together with improved resistance to sorafenib. Ferroptosis is a recently proposed iron-dependent form of cell death characterized by lipid peroxidation and accumulation of reactive oxygen species. High-dose auranofin has been shown to induce ferroptosis by inhibiting TrxR. The role of ferroptosis in NAFLD has been recently studied, and the effects are varied at different stages. Ferroptosis promotes the progression of hepatic steatosis, NASH, and associated fibrosis, while inhibiting the development of cirrhosis and HCC. Auranofin and buthionine sulfoxime co-treatment induced ferroptosis in HCC cell lines. These findings indicate that auranofin could inhibit cell proliferation by regulating cell death in HCC. However, there has been no relevant study suggesting the effects of TrxR inhibition by auranofin in hepatic steatosis and NASH progression. Furthermore, given that ferroptosis may have opposite effects on different stages of NAFLD, future studies are needed to explore the effect of auranofin on ferroptosis in NASH. Whether there is an optimal time point for modulating ferroptosis still requires further exploration, as NASH will eventually progress to HCC.

Overall, the present study demonstrated that auranofin can inhibit NASH progression under experimental conditions, suggesting that it could be a promising repurposed anti-NAFLD agent. Further research is warranted to reveal the mechanisms of auranofin in NAFLD and develop the potential for clinical translation.

Authors’ contribution
Liu YB and Chen MK proposed the idea for the article; Liu YB carried out the literature search, wrote the manuscript, and prepared the language refinement; Chen MK revised the manuscript as the corresponding author and provided comments; and all authors have read and approved the final manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

Abbreviations:
HCC, hepatocellular carcinoma; IRF3, interferon regulatory factor 3; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor kappa B; TrxR, thioredoxin reductase

Letter to the Editor

Letter regarding “Impacts of muscle mass dynamics on prognosis of outpatients with cirrhosis”

Do Seon Song, U Im Chang, and Jin Mo Yang

Department of Internal Medicine, St. Vincent’s Hospital, College of Medicine, The Catholic University of Korea, Suwon, Korea

Keywords: Sarcopenia; Cirrhosis; Prognosis

Dear Editor,

Sarcopenia refers to progressive decline in skeletal muscle, function, and strength with advancing age. Sarcopenia is also highly prevalent in patients with cirrhosis, and the development of sarcopenia in cirrhosis is considered to be associated with systemic inflammation. In addition, sarcopenia has been reported to be associated with adverse clinical outcomes, such as cirrhotic complications, waitlist mortality, and post-transplantation mortality. For these reasons, the importance of assessment of sarcopenia in patients with cirrhosis is being emphasized. However, there are few studies on the association between changes in sarcopenia and the prognosis of cirrhosis. Thus, we read with great interest the article of Kim et al., which described that the change in muscle mass was a good predictor of the development of complication of cirrhosis independent of liver function. However, it should be considered that change in muscle mass may not be an independent prognostic factor after adjusting Child-Pugh and Model for End-stage Liver Disease scores, if stratified by sex.

Secondly, the lifestyle of cirrhotic patients should be considered. In the study of Kim et al., alcohol-related liver disease accounted for 21.0% of all patients, and was the second most common etiology. In addition, alcohol-related liver disease was an independent risk factor for the development of complication. However, there was no description of alcohol use after enrollment. With regard to alcohol-related liver disease, hepatic dysfunction may cause sarcopenia, and alcohol

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can also cause sarcopenia directly or by its metabolites.\textsuperscript{14} Ongoing alcohol use after enrollment might be directly associated with the development of cirrhotic complications. The other lifestyle factors to consider are nutrition and physical activity. Malnutrition is frequently observed in patients with cirrhosis by multifactorial etiologies, such as inadequate dietary intake, ascites, gastroparesis, hormonal change, and altered metabolism.\textsuperscript{15} In addition, reduced exercise capacity and impaired physical performance are commonly observed in patients with cirrhosis.\textsuperscript{16} Physical inactivity might lead to sarcopenia in cirrhotic patients, as physical activity and exercise are anabolic stimuli that can improve the muscle protein balance, reducing the protein loss and increasing the muscle mass and contractile function.\textsuperscript{16} Therefore, it is necessary to consider factors such as alcohol abuse, diets, and physical activity of enrolled patients during the follow-up period.

Thirdly, the quality of muscle is important, as well as muscle mass. Muscle quality is associated with myosteatosis, which refers to ectopic fat infiltration in muscle. Myosteatosis is defined by lower mean skeletal muscle radiodensity on computed tomography (CT), and it is common in cirrhotic patients with prevalence of 16–82%. Myosteatosis is independently associated with mortality and complications in patients with cirrhosis.\textsuperscript{17} Since CT was used for muscle mass evaluation, assessing myosteatosis would also be possible in the patients enrolled in the study by Kim et al.\textsuperscript{11} Moreover, further analysis of myosteatosis would provide additional prognostic information in cirrhotic patients.

In conclusion, we genuinely appreciate the valuable work of Kim et al.,\textsuperscript{11} which demonstrated that the change in muscle mass is an independent prognostic factor in predicting the development of cirrhotic complications. However, consideration of other issues that can affect muscle mass and quality in cirrhotic patients will be more helpful in identifying patients with a poor prognosis.

**Authors’ contribution**

Concept of the work: D.S.S. and J.M.Y.; drafting article: D.S.S. and U.I.C.; critical revision of the article: J.M.Y

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


**Abbreviation:**

CT, computed tomography
Letter to the Editor

Letter regarding “COVID-19 vaccine immunogenicity among chronic liver disease patients and liver transplant recipients: A meta-analysis”

Rujittika Mungmunpuntipantip¹ and Viroj Wiwanitkit²
¹Private Academic Consultant, Bangkok, Thailand; ²Department of Research Center, Chandigarh University, Punjab, India

Dear Editor,

We would like to share ideas on “COVID-19 vaccine immunogenicity among chronic liver disease patients and liver transplant recipients: A meta-analysis.”¹ In contrast to liver transplant recipients, patients with chronic liver disease exhibited a good humoral response to the coronavirus disease 2019 (COVID-19) vaccination, according to Cheung et al.¹ We also agree that COVID-19 is helpful in immunogenicity. The immunological response of a vaccine recipient with an underlying medical condition may be different from that of a healthy individual. Potential confounding factors must be taken into consideration while interpreting the current report. Prior asymptomatic COVID-19 infection is a possibility, and is not unusual.² If there is no method to rule out prior cases of asymptomatic COVID-19, it would be challenging to evaluate the effectiveness of the vaccine.

Authors’ contribution

RM: 50% ideas, writing, approval for submission; VW: 50% ideas, supervision, approval for submission

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES


Abbreviation:

COVID-19, coronavirus disease 2019
Dear Editor,

The euglycemic-hyperinsulinemic clamp (EHC) is the gold standard for assessing insulin sensitivity in peripheral tissues. However, simpler metrics are needed to assess insulin resistance (IR). The metabolic score for the IR (METS-IR) index is a new metric for measuring IR that is simple, reliable, and reproducible. Although Lee et al. applied this score to Koreans, we would like to address some points regarding the associations between non-alcoholic fatty liver disease (NAFLD) and METS-IR.

First, is the METS-IR a reliable score? This score was first proposed by a Mexican research team. The METS-IR discovery sample included 125 subjects who underwent the EHC. The study included subjects aged 20–79 years, with a wide range of body mass indices (18–34.9 kg/m²), who were recruited from the outpatient diabetes clinic of a university hospital in Mexico City. Among the 125 subjects, 68 had type 2 diabetes mellitus (DM) and 57 did not. The subjects with DM were included if they met the following conditions: their glycated hemoglobin concentration was <8%; they did not take insulin; and they were treated with only metformin. However, the number of patients used to develop this score was too small. Furthermore, the composition of the discovery population was heterogeneous. No precise definition of how the METS-IR discovery population was recruited was provided. Although the factors included in the METS-IR score reflect IR, the question is, whether an appropriate patient population was recruited to develop the METS-IR.

Second, NAFLD progresses from simple steatosis, steatohepatitis, and fibrosis to cirrhosis. The authors argued that METS-IR can be used to predict the incidence of NAFLD. As mentioned in the editorial by Kim and Cheong, METS-IR was inversely correlated with the prediction of fibrosis in patients with NAFLD in another study. The same authors explained that a reduction in triglycerides with the progression of liver fibrosis was one hypothesis. Serum triglyceride levels decrease as liver disease progresses to liver fibrosis. As triglyc-

Keywords: Insulin resistance; Non-alcoholic fatty liver disease; Incidence

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Triglycerides are normally the principal source of lipids in the liver, the fat mass in the liver may decrease with fibrosis. Therefore, if METS-IR is strongly affected by triglycerides, this is a major limitation of the METS-IR index. Triglyceride levels can be affected by many factors, including uric acid, being overweight, arterial blood pressure, use of oral contraceptives, consumption of alcohol and tobacco, lack of physical exercise, thyroid disease, and medications, such as diuretics, hormones, corticosteroids, and beta blockers. We can easily find patients with NAFLD, and there are patients with abnormally elevated triglycerides or already taking triglycerides lowering agents, which may limit the use of METS-IR. The authors should explain the mechanism for the contradictory results in predicting steatosis and liver fibrosis with the METS-IR index.

Third, the homeostatic model assessment for insulin resistance (HOMA-IR) contains insulin in the formula, while METS-IR does not include insulin in the formula (Table 1). Additionally, METS-IR includes the blood lipid profile in the formula, while HOMA-IR does not. The METS-IR is superior to the HOMA-IR for predicting incident NAFLD, and is not inferior to the HOMA-IR for predicting prevalent NAFLD. Then, the incidence of NAFLD can be easily detected by images or laboratory findings. If METS-IR is non-inferior for predicting the development of NAFLD, why do we need a more complex formula to predict NAFLD? Another question arises as to whether METS-IR is a more useful marker for early detection and prediction of insulin sensitivity than HOMA-IR. Therefore, it is necessary to consider the clinical use and application of METS-IR in fatty liver patients.

In conclusion, further studies are needed to determine whether METS-IR is an appropriate predictor for NAFLD incidence.

Table 1. Formulas of METS-IR and HOMA-IR

<table>
<thead>
<tr>
<th>Formula</th>
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<tr>
<td>METS-IR: In (2 × FPG [mg/dL] + fasting serum triglyceride [mg/dL]) × BMI (kg/m²) / ln (HDL cholesterol [mg/dL])</td>
</tr>
<tr>
<td>HOMA-IR: (fasting serum insulin [μIU/mL] × FPG [mg/dL] / 405)</td>
</tr>
</tbody>
</table>

METS-IR, metabolic score for the insulin resistance; HOMA-IR, homeostatic model assessment for insulin resistance; FPG, fasting plasma glucose; BMI, body mass index; HDL, high-density lipoprotein.

Conflicts of Interest

The author has no conflicts to disclose.

REFERENCES


Abbreviations:
DM, diabetes mellitus; EHC, euglycemic-hyperinsulinemic clamp; HOMA-IR, homeostatic model assessment for insulin resistance; IR, insulin resistance; METS-IR, metabolic score for the insulin resistance; NAFLD, non-alcoholic fatty liver disease.
Letter to the Editor

Reply to Letter regarding “Auranofin attenuates hepatic steatosis and fibrosis in nonalcoholic fatty liver disease via NRF2 and NF-κB signaling pathways”

Seung Min Lee¹ and Dae Won Jun²

¹Department of Translational Medicine, Graduate School of Biomedical Science & Engineering, Hanyang University, Seoul; ²Department of Internal Medicine, Hanyang University Hospital, Hanyang University College of Medicine, Seoul, Korea

Keywords: Auranofin; Ferroptosis; Non-alcoholic fatty liver disease; Hepatocellular carcinoma

Dear Editor,

We appreciate your interest in our study. As pointed out by Liu and Chen,¹ non-alcoholic fatty liver disease (NAFLD) has a broad heterogeneous spectrum and a diverse pathophysiology.²⁻⁸ The relationship between auranofin-induced ferroptosis and NAFLD is somewhat complex.⁹ It depends on the cell type and disease condition. Ferroptosis is associated with the pathogenesis of NAFLD, and inhibiting ferroptosis can inhibit necrotic cell death, inflammatory cell infiltration, and inflammatory cytokine expression in early-stage NAFLD.⁹ However, in late-stage NAFLD and hepatocellular carcinoma, inhibition of ferroptosis is associated with disease progression.¹⁰,¹¹ In previous studies, the expression of glutathione peroxidase (GPX) 4, which protects cells against membrane lipid peroxidation, has been shown to vary according to the severity of NAFLD. In addition, the association between ferroptosis and NAFLD has been observed to vary depending on the animal model of NAFLD. This indicates that ferroptosis may play various roles at different stages of NAFLD. System Xc⁻ and NAFLD also share a complex relationship. A large body of evidence suggests that auranofin induces ferroptosis via the cystine-glutamate antiporter system Xc⁻. Auranofin has been shown to induce ferroptosis via the GSH/GPX axis. Additionally, our previous study indicated that auranofin inhibited system Xc⁻ in macrophages and the NOD-like receptor family pyrin domain containing 3 inflammasome in inflammatory cells.¹² However, ferroptosis can simultaneously induce iron-dependent lipid peroxidation. Yang et al.¹³ demonstrated that auranofin at high doses (25 mg/kg) induces ferroptosis but causes lipid peroxidation by inhibiting thioredoxin reductase activity. In conclusion, it is evident that auranofin acts as an inhibitor of system Xc⁻. However, ferroptosis induced by system Xc⁻ inhibitors appears to play a different role in disease progression depending on the liver cell type and severity of NAFLD. Therefore, for the clinical application of auranofin, it is important to select a target population that is anticipated to have a positive therapeutic effect.

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Authors’ contribution
All authors contributed in conception of the work and drafting of the article. All authors provided final approval of the version to be published.

Conflicts of Interest
The authors have no conflicts to disclose.

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2. Kim HY. Recent advances in nonalcoholic fatty liver disease metabolomics. Clin Mol Hepatol 2021;27:553-559.

Abbreviations:
GPX, glutathione peroxidase; NAFLD, non-alcoholic fatty liver disease
Letter to the Editor

Reply to Letter regarding “Impacts of muscle mass dynamics on prognosis of outpatients with cirrhosis”

Tae Hyung Kim, Young Kul Jung, and Hyung Joon Yim
Department of Internal Medicine, Korea University Ansan Hospital, Ansan, Korea

Keywords: Sarcopenia; Prognosis; Liver cirrhosis; Risk factors; Lifestyle

Dear Editor,

We sincerely appreciate the letter from Song et al.1 for contemplating our recent paper on the prognostic impact of muscle mass change in outpatients with cirrhosis, published in Clinical and Molecular Hepatology.2 We agree that sex and lifestyle influence muscle mass and that myosteatosis is an important prognostic marker in patients with cirrhosis. Many diagnostic criteria for sarcopenia have been established based on sex-specific differences, and this study was conducted by adopting one of them. However, the change in muscle mass may differ from the muscle mass itself. In our study, change in muscle mass (ΔSMI)/yr%, which represented the change in muscle mass, was not an absolute value but a rate value divided by the muscle mass at inclusion. Thus, regardless of sex, the impact of ΔSMI/yr% could be consistent.

The analysis of subgroup according to sex, which was not presented in the original paper, revealed that ΔSMI/yr% continued to be an independent predictor for the development of cirrhosis complications in men even after adjusting for the model for end-stage liver disease score. In the case of women, the correlation marginally significant (Table 1), which might be attributed to the fact that women in our cohort were fewer and had a lower incidence of cirrhosis complications than men. In addition, every 1-point increase in ΔSMI/yr% was associated with a 5.4% and 4.0% reduction in the risk of cirrhosis complications in men and women, respectively.

Of course, there were other results suggesting sex-specific

Table 1. Multivariable cox-regression analyses for the development of LC complication after 1-year CT according to sex

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSMI/yr%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n=381)</td>
<td>0.946</td>
<td>0.917–0.976</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women (n=214)</td>
<td>0.960</td>
<td>0.921–1.001</td>
<td>0.055</td>
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</tbody>
</table>

Results were derived after adjusting for age and model for end-stage liver disease score. LC, liver cirrhosis; CT, computed tomography; HR, hazard ratio; CI, confidence interval; ΔSMI, change in muscle mass.

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

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Continuous alcohol consumption is an obvious aggravating factor for muscle loss, decompensation, and mortality in patients with alcohol-related liver disease.\(^3\)\(^-\)\(^6\) In addition, abstinence is a fundamental treatment with long-term benefits, including a 10–30% reduction in mortality.\(^4\)\(^,\)\(^7\)\(^,\)\(^8\) Thus, drinking behavior after inclusion could be an important factor in the prognosis of patients with cirrhosis, as mentioned by Song et al.\(^1\) However, drinking behavior is highly variable, and there is no standardized method for measuring it, making it difficult to incorporate into research.\(^9\) Malnutrition and insufficient physical activity are well-known risk factors for sarcopenia and the prognosis of patients with cirrhosis. Conversely, a well-controlled diet and regular exercise could improve sarcopenia even in patients with cirrhosis.\(^3\)\(^,\)\(^10\)\(^-\)\(^12\)

Several pioneering studies have shown that myosteatosis is significantly associated with decompensation, hepatocellular carcinoma development, and mortality in patients with chronic liver disease.\(^13\)\(^-\)\(^15\) Even in these studies, it was unclear whether myosteatosis was a better prognostic predictor than sarcopenia. However, it appeared to be a prognostic predictor acting independently of sarcopenia.\(^15\)

Myosteatosis and lifestyle, including drinking behavior, nutrition, and physical activity, are now considered essential in sarcopenia-related studies. However, as mentioned in the limitations of the original paper, the protocol in our cohort did not include measurements for these factors. Therefore, further studies are required to address these factors.

**Authors’ contribution**

Concept of the work: Y.K.J. and H.J.Y.; drafting article: T.H.K.; critical revision of the article: Y.K.J.

**Acknowledgments**

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**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


**Abbreviation:**

ΔSMI, change in muscle mass

et al. Muscle alterations are associated with minimal and overt hepatic encephalopathy in patients with liver cirrhosis. Hepatology 2019;70:1704-1713.


Dear Editor,

We would like to thank Mungmunpuntipantip and Wiwanitkit for their response to our recent meta-analysis on the possible confounding factors of vaccine recipients, including underlying medical conditions and prior coronavirus disease 2019 (COVID-19) infection. COVID-19 infection can cause not only gastrointestinal symptoms but also hepatic injury, including cholangitis and autoimmune hepatitis. Therefore, vaccination is of paramount importance to prevent COVID-19 infection and its disease severity.

We agree that patients with more comorbidities may have different vaccine immunogenicity compared to healthy individuals. Table 1 shows the list of comorbidities among patients included in our analysis. No study provided individual data for the outcome of seroconversion regarding the presence of comorbidity. Importantly, non-alcoholic fatty liver disease, which may affect vaccine immunogenicity, is also associated with a higher risk of comorbidities, such as diabetes mellitus. Unlike the etiology of liver disease and cirrhosis status, wherein we used a prevalence of 80% as the cut-off for classification, the data for comorbidity were heterogeneous and no cut-off could be drawn. Hence, we could not perform subgroup analysis with respect to each comorbidity. In addition, certain medications (e.g., antibiotics, angiotensin-converting enzyme inhibitors, histamine 2 receptor antagonists) may affect either the COVID-19 vaccine response or disease severity. However, drug data were lacking in the included studies.

Although prior history of COVID-19 infection was part of...
<table>
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<th>Study</th>
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Values are presented as number (%).
the exclusion criteria in our study, asymptomatic cases might have been enrolled, as the baseline levels of antibodies were measured. Timmermann et al.\textsuperscript{9} found 2/120 asymptomatic patients with positive anti-nucleocapsid-immunoglobulin G antibodies, and these patients were excluded from subsequent analysis. A meta-analysis revealed that 0.25% of the tested general population were asymptomatic infections.\textsuperscript{10} Nevertheless, this issue may not have a significant impact on our results due to a large sample size of 3,945 patients. It is noteworthy that vaccinated recipients, especially liver transplant patients, should still adhere to other infection prevention and control measures, such as social distancing.\textsuperscript{11}

**Authors’ contribution**

Ka Shing Cheung and Chiu Hang Mok were involved in data retrieval, statistical analysis and drafting of the manuscript. Wai Kay Seto and Man Fung Yuen were involved in supervision.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**REFERENCES**


Dear Editor,

We appreciate Dr. Lee’s interest in this study. As Dr. Lee commented in the letter, the metabolic score for insulin resistance (METS-IR) has been used as a simple, reliable, and reproducible surrogate insulin resistance (IR) marker in the South American population.\(^1\) However, several Korean epidemiological studies using METS-IR\(^2\)\(^-\)\(^6\) lack validation and cut-off points that would help to identify IR in Koreans. A follow-up study is needed to compare METS-IR with the hyperinsulinemic-euglycemic index in order to determine whether it reflects insulin sensitivity well and if it is more reliable than the homeostatic model assessment for IR (HOMA-IR) used in the general Korean population.

The formula that constitutes METS-IR uses triglyceride levels, which are affected by multiple factors, including arterial blood pressure, alcohol use, carbohydrate intake, and use of medications such as diuretics and oral contraceptives.\(^7\) We adjusted for hypertension, alcohol use, and energy intake because such factors also contribute to nonalcoholic fatty liver disease (NAFLD).\(^8\)\(^-\)\(^10\) However, the lack of information in the Korean Genome and Epidemiological Study dataset about specific medication use could serve as a potential confounder in our study.\(^11\) Despite this limitation, there is also the pos-
sibility that the potential effect of the confounder was attenuated because we used community-based cohort data to analyze a large population. Further clinical trials should be performed with controlling for potential confounding variables to verify the association between METS-IR and NAFLD.

Current guidelines for management of NAFLD state that radiologic methods, such as abdominal ultrasonography, controlled-attenuated parameter, or unenhanced abdominal computed tomography, are acceptable to diagnose hepatic steatosis. Serologic surrogate markers, such as NAFLD-liver fat score, hepatic steatosis index, or fatty liver index, can be used to assess hepatic steatosis if radiological examinations are infeasible. We believe that METS-IR will be less accurate for diagnosis of hepatic steatosis than radiologic tests or serologic surrogate markers for hepatic steatosis. This is because METS-IR was developed as a surrogate marker for IR. We do not claim to use METS-IR as a single predictive model for NAFLD; if fatty liver disease has not yet developed, abdominal ultrasonography or abdominal computed tomography will not provide additional information about the risk of developing NAFLD. As IR is closely related to NAFLD, we believe that the assessment and management of IR are important strategies for the early prevention and management of NAFLD. In clinical practice, HOMA-IR is the most commonly used surrogate marker for IR. However, serum insulin level is not routinely measured in the general clinical field, so METS-IR can be applied more easily than HOMA-IR even though METS-IR uses a more complex formula. In our study, METS-IR was not inferior to HOMA-IR in predicting the prevalence of NAFLD, and it was superior to HOMA-IR in predicting the incidence of NAFLD. Therefore, our findings suggest that METS-IR can be used as an IR marker in patients with or who are at risk of developing NAFLD. Further experimental studies and clinical trials should be performed to elucidate the mechanism by which METS-IR is positively related to NAFLD and negatively related to advanced liver fibrosis, considering the changes in METS-IR values over time. Further studies on the genetic variations affecting METS-IR values, hepatic steatosis, and liver fibrosis are also necessary.

**Authors’ contribution**

Study concept and design: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn; Data collection: Jun-Hyuk Lee, Kyongmin Park, Hye Sun Lee, and Hoon-Ki Park; Data analysis and interpretation: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn; Manuscript writing: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn. Final approval of the manuscript: All authors

**Conflicts of Interest**

The authors have no conflicts to disclose.

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**Abbreviations:**

HOMA-IR, homeostatic model assessment for insulin resistance; IR, insulin resistance; METS-IR, metabolic score for insulin resistance; NAFLD, non-alcoholic fatty liver disease


Dear Editor,

We appreciate the interest and comments from Kim et al. on our recently published paper on the impact of nationwide hepatocellular carcinoma (HCC) surveillance on the prognosis of patients with chronic liver disease. In the article, the authors speculated on the current status of HCC surveillance and suggested a course of action. We agree with the suggestions and would like to discuss several related issues in detail.

With the current era of antiviral treatment in chronic hepatitis B (CHB) or chronic hepatitis C (CHC), high-risk groups for HCC surveillance need to be redefined in a more detailed manner, especially considering the use of antiviral agents in chronic viral hepatitis. Based on the annual incidence of HCC and its cost-effectiveness, HCC surveillance is traditionally recommended for patients with CHB aged >40 years or with any type of cirrhosis. However, the risk of HCC development in CHB has changed since the introduction of antiviral treatment. The risk of HCC development is decreased in CHB patients receiving nucleotide analogs as the use of potent antiviral treatment effectively suppresses hepatitis B virus replication. However, the risk of HCC development remains higher in these patients than in those with inactive CHB. As a result, HCC surveillance in CHB should be subdivided based on the phases of CHB (such as, immune-tolerant, immune-active phase with the use of antiviral treatment, and inactive phase).

Additionally, the reduced risk of advanced fibrosis or cirrhosis during or after antiviral therapy should be considered. Recent studies have reported that long-term use of antiviral agents could induce the regression of advanced fibrosis or cirrhosis in CHB. It is well established that cirrhosis is a crucial factor in the development of HCC. In a certain percentage of patients, regression of advanced fibrosis or cirrhosis fol-
lowing antiviral treatment has been reported. Therefore, it is necessary to consider the regression of advanced fibrosis or cirrhosis as a risk factor for HCC development in CHB patients receiving antiviral treatment. Similarly, information regarding HCC surveillance in patients with CHC, who have achieved sustained virologic response (SVR) after direct-acting antivirals is unclear. Therefore, it is necessary to define risk stratification for HCC surveillance in this group of patients. As mentioned earlier, the high-risk group for HCC surveillance is defined based on the annual incidence of HCC and cost-effectiveness. Unfortunately, our study lacked data on cost-effectiveness, such as incremental cost-effectiveness ratio or quality-adjusted life year. A recent meta-analysis has reported that biannual surveillance for HCC in CHC patients who achieved SVR is cost-effective for patients up to 70 years old with cirrhosis and up to 60 years old with advanced fibrosis.

It is also unclear whether HCC surveillance is helpful for survival gain in patients with nonalcoholic fatty liver disease (NAFLD), particularly those without advanced fibrosis or cirrhosis. NAFLD-associated HCC can frequently occur without advanced fibrosis or cirrhosis, even in patients with simple steatosis and without steatohepatitis. Therefore, it is necessary to define a high-risk group for NAFLD-associated HCC by another method apart from advanced fibrosis or cirrhosis. A recent study showed that a genetic polymorphism might be a significant risk factor for NAFLD-associated HCC. A polygenic risk score based on genetic polymorphisms associated with hepatic fat may also prove to be a useful tool to stratify high-risk groups for NAFLD-associated HCC, particularly in patients without advanced fibrosis or cirrhosis.

Screening is the next issue that needs to be addressed regarding HCC surveillance. Although ultrasonography is recommended as a screening tool, the diagnostic accuracy of this method for early-stage HCC is suboptimal in some patients. Furthermore, the diagnostic accuracy in obese patients is lower than that in non-obese patients. Additionally, it is difficult to differentiate HCC from regenerative nodules or dysplastic nodules in patients with cirrhosis using this method. Therefore, an alternative screening tool beyond ultrasonography is needed to increase the efficacy of HCC surveillance. Computed tomography (CT) or magnetic resonance imaging (MRI) has shown better performance in the detection of HCC in cirrhotic patients compared to ultrasonography. However, it is necessary to clarify the role of CT or MRI as HCC surveillance tools based on their adverse effects and costs.

Taken together, it can be concluded that HCC surveillance in patients with chronic liver disease is crucial for detecting early-stage tumors and improving overall survival. However, changes are needed regarding the strategies being employed for HCC surveillance. The use of antiviral agents in viral hepatitis, dynamic changes in advanced fibrosis or cirrhosis, genetic factors for HCC development in non-cirrhotic patients, particularly in NAFLD, and screening tools other than ultrasonography should be considered to achieve better precision in HCC surveillance among patients with chronic liver disease.

Authors’ contribution
All authors contributed to the conception of the study and drafting of the manuscript. All authors contributed to the critical revision of this article. All the authors provided final approval for the version to be published.

Conflicts of Interest
The authors have no conflicts to disclose.

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Abbreviations:
CHB, chronic hepatitis B; CHC, chronic hepatitis C; CT, computed tomography; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; SVR, sustained virologic response


Correspondence on Editorial regarding “Screening and prediction of nonalcoholic fatty liver disease using a peripheral insulin resistance index: Potential benefits and limitations”

Jun-Hyuk Lee, Kyongmin Park, Hye Sun Lee, Hoon-Ki Park, Jee Hye Han, and Sang Bong Ahn

Keywords: Insulin resistance; Non-alcoholic fatty liver disease; METS-IR; HOMA-IR

Dear Editor,

We appreciate Drs. Soon Sun Kim and Jae Youn Cheong’s valuable comments on our research determining predictive values of two insulin resistance (IR) indices, metabolic score for IR (METS-IR) and homeostatic assessment model for IR (HOMA-IR), for the prevalence and incidence of non-alcoholic fatty liver disease (NAFLD), published in Clinical and Molecular Hepatology. We are happy to respond to the points they raised in their editorial letter.

The prevalence of NAFLD has steadily increased and is estimated to be approximately >50% by 2040. As NAFLD is a risk factor for morbidities such as atherosclerotic cardiovascular disease or dementia, many efforts to identify markers for early detection and prediction of NAFLD have been made. The association between NAFLD and metabolic dysfunction has been extensively investigated. The established risk factors for NAFLD include obesity, hypertriglyceridemia, metabolic syndrome, and diabetes mellitus. Although these factors are closely related to peripheral IR, a previous study has demonstrated that peripheral IR, not hepatic IR, correlates with hepatic fat. Thus, we hypothesized that an index that reflects peripheral IR would also be appropriate for predicting the prevalence and incidence of NAFLD. The METS-IR demonstrated similar predictive power for the prevalence of NAFLD as HOMA-IR and superior predictive power for the incidence of NAFLD compared to HOMA-IR. These results may be due to the high METS-IR reflecting the presence of meta-
bolic syndrome and/or diabetes mellitus. Although we used only the baseline METS-IR value in this study, the value of METS-IR changes with time. The longitudinal data of patients we encountered in clinical practice will provide useful information if the pattern of the METS-IR change with time is related to NAFLD. Therefore, we plan to verify the associations between METS-IR trajectories and incident NAFLD in a future study.

In a very recent study, the predictive power for incident NAFLD of the METS-IR at 4 years in Chinese individuals without obesity was higher than that in our study (time-dependent area under the receiver-operating-curve [AUROC] in the Chinese study vs. our study, 0.752 vs. 0.683). Different ethnicities, short follow-up periods, and the use of abdominal ultrasonography to define NAFLD could have contributed to the discordance in the results between the two studies. Because abdominal ultrasonography was not performed in the Korean Genome and Epidemiology Study (KoGES), we used NAFLD-liver fat scores ≥36 to define NAFLD in our study. In accordance with the editors’ comments, we additionally performed the same analysis using a hepatic steatosis index (HSI) ≥36 to define NAFLD for validation. Among 8,360 participants (35.0%) developed NAFLD during the 13.5-year follow-up period. The time-dependent AUROC for the incidence of NAFLD using HSI decreased compared to those of NAFLD using the METS-IR-liver fat score. In particular, the predictive power of the HOMA-IR decreased from 0.831 (0.821–0.842) to 0.544 (0.529–0.559), whereas that of METS-IR decreased from 0.831 (0.821–0.842) to 0.717 (0.705–0.730). Additionally, among 5,670 participants without NAFLD at the baseline survey, 2,111 participants (25.3%) had NAFLD. The predictive powers of the METS-IR and HOMA-IR for the prevalence of NAFLD using HSI decreased compared to those of NAFLD using the METS-IR-liver fat score. In particular, the predictive power of the HOMA-IR decreased from 0.831 (0.821–0.842) to 0.544 (0.529–0.559), whereas that of METS-IR decreased from 0.831 (0.821–0.842) to 0.717 (0.705–0.730). Additionally, among 5,670 participants without NAFLD at the baseline survey, a total of 1,985 participants (35.0%) developed NAFLD during the 13.5-year follow-up period. The time-dependent AUROC for the incidence of NAFLD in HOMA-IR decreased from 0.551 (0.539–0.563) to 0.522 (0.514–0.530), whereas that of METS-IR decreased from 0.683 (0.671–0.695) to 0.575 (0.565–0.585).

Although both NAFLD-liver fat score and HSI are highly reliable markers for predicting NAFLD and NAFLD is closely related to IR, the correlations of these two markers with the IR indices were heterogeneous in this study. Similarly, the correlation coefficient between the NAFLD-liver fat score and METS-IR (r1) was 0.499, and that between the HSI and HOMA-IR (r2) was 0.439, with significant differences between r1 and r2. While the correlation coefficient between the NAFLD-liver fat score and HOMA-IR (r3) was 0.650, the correlation coefficient between the HSI and HOMA-IR (r4) was only 0.078, with significant differences between r3 and r4. These results suggest a possible information bias. To determine a more precise result, defining NAFLD using imaging studies such as abdominal ultrasonography or abdominal computed tomography need to be considered in future studies.

In another study, we compared the predictive power of the METS-IR/triglyceride-glucose (TyG) index/HOMA-IR for advanced liver fibrosis in patients with NAFLD using the KoGES dataset. The METS-IR had the highest predictive power for the prevalence of advanced liver fibrosis, followed by the TyG index and HOMA-IR. Although both the METS-IR and TyG index were significantly associated with incident advanced liver fibrosis in the crude model, the significant association with advanced liver fibrosis was maintained only in the METS-IR, not the TyG index, in the adjusted model. While the TyG index only reflects lipid profile, the METS-IR reflects both the lipid profile and obesity. The difference between these two IR indices made METS-IR better reflect malnutrition in patients with advanced liver disease, which may have caused METS-IR to have a significant association with advanced liver fibrosis in the adjusted model. Considering the inverse relationship between the METS-IR at baseline and incident advanced liver fibrosis in patients with NAFLD, improvement in the METS-IR does not always indicate an improvement in NAFLD. However, we cannot guarantee that changes in the METS-IR are also related to advanced liver fibrosis because both the lipid profile and body mass index fluctuate with time. Hence, determining whether the trajectories of different IR indices are associated with advanced liver fibrosis is necessary.

In conclusion, a high METS-IR can predict the prevalence and incidence of NAFLD. However, this does not always indicate that a low METS-IR score predicts improvement in NAFLD. Therefore, the current evidence for using METS-IR to monitor patients with NAFLD is insufficient. Follow-up studies should be performed to determine whether changes in

**Abbreviations:**
- AUROC, area under the receiver-operating-curve
- HSI, hepatic steatosis index
- HOMA-IR, homeostatic assessment model for insulin resistance
- IR, insulin resistance
- KoGES, Korean Genome and Epidemiology Study
- METS-IR, metabolic score for insulin resistance
- NAFLD, non-alcoholic fatty liver disease
- TyG, triglyceride-glucose
various IR indices over time are related to NAFLD and/or liver fibrosis.

**Authors’ contribution**

Study concept and design: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn; Data collection: Jun-Hyuk Lee, Kyongmin Park, Hye Sun Lee, and Hoon-Ki Park; Data analysis and interpretation: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn; Manuscript writing: Jun-Hyuk Lee, Jee Hye Han, and Sang Bong Ahn. Final approval of the manuscript: All authors

**Conflicts of Interest**

The authors declare no conflicts of interest.

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Correspondence on Editorial regarding “Baveno-VII criteria to predict decompensation and initiate non-selective beta-blocker in compensated advanced chronic liver disease patients”

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Keywords: Liver cirrhosis; Hypertension; Portal; Ascites; Hepatic encephalopathy

Dear Editor,

We would like to thank Dr. Semmler and colleagues1 for their interest in our study. In the present study, we sought to demonstrate the applicability of non-invasive tests (NIT)-based criteria in risk-stratifying compensated cirrhosis patients in the real-world clinical practice.

We included patients with cirrhosis driven by non-alcoholic steatohepatitis or non-alcoholic fatty liver disease, whom etiological cure is not currently available - these patients represent compensated advanced chronic liver disease (cACLD) without removal of primary etiology. We also included viral-related cirrhosis with adequate virological suppression, which is the current standard of care.3,4 The inclusion of treated viral-related cirrhosis should not invalidate our conclusion, because even after virological suppression, cirrhosis patients with clinically significant portal hypertension (CSPH) may still have CSPH, meaning these patients remain at risk of future decompensation and hepatocellular carcinoma.2,5

The number needed-to-treat was probably higher by including patients with treated viral-related cirrhosis in this study. However, given the robust scientific evidence of removing primary etiology can improve outcomes in viral-related cirrhosis, there should be little debate on whether these patients should be treated.6 What remains uncertain, is whether non-selective beta-blockers (NSBB) are needed to prevent decompensation in all cACLD patients with virological suppression and persistence CSPH, when CSPH was assessed using NIT-based criteria.7 Indeed, there was a risk of first hepatic decompensation in almost half of our patients (predominantly cured hepatitis C virus [HCV] infection and CSPH-ruled in by NIT) vs. the placebo group of the PREDESCI (β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension) trial,

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which mostly included untreated HCV patients with CSPH (13.3% vs. 24.0%). This difference likely reflects the impact of virological suppression in HCV patients in our cohort. In a way, it is reassuring to see that NIT-based assessment of CSPH remained predictive of liver decompensation in viral-related cirrhosis patients achieving viral suppression. Since cirrhosis patients may continue to have CSPH (thus, the risk of liver decompensation), NSBB should be considered to prevent decompensation in these patients.

As described in our study, patients with NSBB at baseline (presumably at higher baseline risk of CSPH, high-risk varices and liver decompensation) were excluded since NSBB may reduce the risk of liver decompensation, as shown in PREDESCI trial. Nevertheless, this subgroup was small, and subgroup analysis showed that CSPH (liver stiffness measurement ≥25 kPa) remained predictive of decompensation after excluding patients with high-risk varices.

There were a significant proportion of patients falling within the grey zone, which was also demonstrated in a recent study by Semmler et al. This is consistent with the performance of transient elastography to exclude or include patients with advanced fibrosis. Unfortunately, we did not have the data on spleen stiffness and the ratio of von Willebrand factor and platelet count (VITRO) in the current cohort. Finally, as stated in our manuscript, we performed competing risk regression by cluster to account for heterogeneity and regional differences across the four cohorts of patients.

In summary, our findings demonstrated that Baveno-VII criteria of CSPH can predict liver decompensation and liver-related events in compensated cirrhosis/cACLD patients. We agree that a pragmatic “non-invasive” PREDESCI trial would be desirable to re-ensure our current clinical practice using contemporary patients, particularly cACLD patients after HCV cure, as it would also help confirm our findings. Until then, our findings suggest that NSBB should be considered in cirrhosis patients with CSPH diagnosed using non-invasive criteria.

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**Conflicts of Interest**

YJW is an invited speaker for Gilead Science and AbbVie. The other authors have no conflicts to disclose.

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**Abbreviations:**
cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; HCV, hepatitis C virus; NASH, non-alcoholic fatty liver disease; NIT, non-invasive tests; NSBB, non-selective beta blocker; VITRO, the ratio of von Willebrand factor and platelet count.

Correspondence on Editorial regarding “HBV pgRNA and HBcrAg reductions at week 4 predict favourable HBsAg response upon long-term nucleos(t)ide analogue in CHB”

Lung-Yi Mak1,2, Wai-Kay Seto1,2, and Man-Fung Yuen1,2
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Keywords: Biomarkers; Treatment outcome

Dear Editor,

We sincerely appreciate the editorial piece from Liang et al. reviewing our recent paper on the role of early on-treatment decline in viral biomarkers in predicting favourable hepatitis surface antigen (HBsAg) response in chronic hepatitis B (CHB) infection, published in Clinical and Molecular Hepatology. We agree with Liang and co-authors on the potential use of hepatitis B core-related antigen (HBcrAg) and hepatitis B virus (HBV) pre-genomic RNA (pgRNA) in multiple facets of management in the clinical context of CHB infection. Our study provided serum-liver correlations in the magnitude of decline in viral biomarkers upon nucleos(t)ide analogue (NA) treatment—those with ≥1 log decline in covalently closed circular DNA (cccDNA) at week 48 had more significant reductions in serum pgRNA and HBcrAg at multiple timepoints of assessment. This further strengthens the proposition for these serum viral biomarkers to be used as surrogates for cccDNA activity.

The findings of our study suggest that subjects without early biomarker response (defined as week 4 pgRNA decline ≥5.32 log copies/mL for hepatitis B envelope antigen (HBeAg)-positive subjects, or week 4 HBcrAg decline ≥2.05 log U/mL for HBeAg-negative subjects) had a low likelihood of achieving favourably low levels of quantitative HBsAg (qHBsAg) (<100 IU/mL) or HBsAg seroclearance, and they should be prioritized for clinical trials while maintaining the NA therapy. As most current trials only consider qHBsAg and/or HBV DNA when screening patients for enrolment eligibility, HBcrAg and pgRNA would provide additional layer of information to identify patients who are most in need for new treatment approaches.

HBsAg seroclearance plus HBV DNA undetectability >6 months after treatment cessation is the primary endpoint for phase III trials in the functional cure program of CHB. Notably, the benchmark of ≥30% patients achieving this endpoint has not been met by any of the currently developing novel compounds, despite initial promising results in qHBsAg knockdown by RNA interference-based therapy. This has engendered discussions about the practicability of such stringent treatment endpoint. Taking a step...
back, a ‘looser’ endpoint of achieving serum qHBsAg <10 IU/mL or <100 IU/mL (HBsAg cut-off levels still subjected to debate) by novel compounds might be more feasible, as such endpoint implies that a patient with CHB had a lower risk of off-therapy virological relapse and can potentially employ the ‘stop-to-cure’ approach to induce functional cure.\(^8\)

The potential of serum HBCrAg and HBV RNA should not be limited to the context of novel compound development, but may also be applicable to consideration of NA withdrawal in those fulfilling criteria.\(^9\) The timing of biomarker assessment relative to NA therapy is an interesting point to consider. Our study looked at the early (as early as 4 weeks) on-treatment viral biomarker profiles instead of end-of-treatment (EOT) levels. The role of EOT pgRNA and/or HBCrAg in off therapy virological control have been investigated in multiple trials.\(^10,11\) Instead of having to wait for reaching EOT (≥3 years, which is the minimum consolidation period for NA in HBeAg-negative patients),\(^12\) early on-treatment profile of these biomarkers would provide valuable insights to identify patients potentially suitable for this treatment approach.

In summary, our study demonstrated that the degree of cccDNA silencing is the main determining factor for favourable HBsAg response, and can be reflected by early on-treatment changes in HBCrAg and HBV RNA. Patients without early biomarker response while on NA, as an additional consideration on top of qHBsAg levels, should be prioritized to participate in clinical trials in order to achieve functional cure.

**Authors’ contribution**

LYM: literature review and original drafting; WKS and MFY: critical revision of article.

**Conflicts of Interest**

LYM serves as advisor for Gilead Sciences. WKS received speaker’s fees from AstraZeneca and Mylan, is an advisory board member of CSL Behring, is an advisory board member and received speaker’s fees from AbbVie, and is an advisory board member, received speaker’s fees and researching funding from Gilead Sciences. MFY serves as advisor/consultant for AbbVie, Assembly Biosciences, Aligos Therapeutics, Arbutus Biopharma, Bristol Myer Squibb, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, Hoffmann-La Roche and Springbank Pharmaceuticals, Vir Biotechnology and receives grant/research support from Assembly Biosciences, Aligos Therapeutics, Arrowhead Pharmaceuticals, Bristol Myer Squibb, Fujirebio Incorporation, Gilead Sciences, Immunocore, Merck Sharp and Dohme, Hoffmann-La Roche, Springbank Pharmaceuticals and Sysmex Corporation.

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**Abbreviations:**

cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; EOT, end-of-treatment; HBCrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis surface antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; pgRNA, pre-genomic RNA; qHBsAg, quantitative HBsAg


Epidemiology and updated management for autoimmune liver disease

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**Epidemiology**

- **AIH**
  - 50s~60s
  - Incidence: 1.37/100,000/year (Global)
  - Prevalence: 17.44/100,000 (Global)
  - Female dominance
  - Lymphoplasma cell infiltration

- **PBC**
  - 50s~60s
  - Incidence: 4.3/100,000/year (USA)
  - Prevalence: 39.2/100,000 (USA)
  - Female dominance
  - Non-suppurative destructive cholangitis

- **PSC**
  - Prevalence: 4.15–18.2/10,000 (Western)
  - 0.05–100,000 (Japan)
  - 50–80% with UC
  - Onion skin fibrosis

**Management**

- **Acute/chronic AIH**
  - Prednisolone
  - MMF
  - Tacrolimus
  - Cyclosporine

- **AS-AIH**
  - High dose prednisolone

- **ALF-AIH**
  - Liver transplantation

- **PBC**
  - 1st line: High dose UDCA
  - 2nd line: Obeticholic acid

- **PSC**
  - No effective medical treatment
  - ERCP: cholangitis with dominant stricture
  - Surveillance for cholangiocarcinoma and UC

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Aberrant activation of autoimmunity may influence the bad impact on the liver. The representative entities of autoimmune liver disease include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC).

AIH is the hepatocellular damage with the infiltration of lymphocyte and plasma cells, which might be induced by uncontrolled autoreactive CD4 and CD8 T cells. Although AIH might be characterized by elevated serum aminotransferase and immunoglobulin G (IgG), and the detection of autoantibodies, the concept of autoimmunity should be confirmed by the exclusion of other liver diseases. The global annual incidence rate of AIH was 1.37 per 100,000 persons, and similar among the Asian, European, and American populations. The ratio of male to female gender was shown as 1:5. The global prevalence rate of AIH was 17.44 per 100,000 persons with a high level of regional variation. The average age of the onset of AIH is the mid-50s.

The strategy of management of AIH is recommended according to disease subtypes such as chronic or acute AIH, acute severe AIH, and acute liver failure (ALF)-AIH. In chronic or acute AIH, glucocorticoid monotherapy or glucocorticoid plus azathioprine could be applied. If remission induction accomplishes, glucocorticoid is tapered to the effective lowest dose or withdrawal, then maintenance therapy with azathioprine ± low dose glucocorticoid should continue. A recent study suggested that a prolonged complete biochemical response which is defined as normalization of aminotransferase and IgG for at least 2 years might be used to stop treatment without liver biopsy. In case of incomplete biochemical response, treatment failure, or drug intolerance, 2nd line treatment including mycophenolate mofetil, tacrolimus, cyclosporin A, or infliximab could be used. In acute severe AIH, high-dose glucocorticoid monotherapy could be applied without azathioprine due to potential hepatotoxicity. At last, in ALF-AIH with encephalopathy, initial evaluation of liver transplantation (LT) should be considered without anticipation of the response to glucocorticoid. While thiopurine methyltransferase activity test is encouraged to avoid azathioprine-related toxicity in European and African descendants, nudix hydrolase 15 variant is associated with the adverse effect of azathioprine in the East Asian population.

PBC is a chronic cholestatic autoimmune liver disease characterized by the slowly progressive destruction of small intrahepatic bile ducts. The incidence and prevalence vary according to the region. PBC presented less frequently in Eastern than in Western countries. The annual incidence in USA and Korea was 4.3, and 0.86 per 100,000 persons, respectively. The prevalence in USA and Korea was 39.2, and 4.75 per 100,000 persons, respectively. PBC occurs most frequently in women in their 50s and 60s. Diagnosis of PBC is based on the three characteristic findings such as serum alkaline phosphatase elevation, positive anti-mitochondrial antibody, and non-suppurative destructive cholangitis in liver biopsy.

The management of PBC is composed of resolving cholestasis and control of its complications. High-dose ursodeoxycholic acid (UDCA) has been approved as a 1st-line therapy, which has dramatically modified the natural course of PBC. However, about 20–30% of patients with PBC showed incomplete response to it. Recently, obeticholic acid presented approximately 50% of treatment response among the incomplete responders to UDCA, where it received accelerated US Food and Drug Administration approval. However, follow-up phase 3 trial (The Clinical Outcomes with OBeticholic Acid in Liver Treatment [COBALT] study) was terminated early due to feasibility challenge in 2021. Therefore, the availability of obeticholic acid is currently limited. Also, add-on of bezafibrate to UDCA in incomplete responders to UDCA significantly improved liver biochemistry and liver stiffness, and LT-free survival. Complications of PBC include fatigue, pruritus, osteoporosis, hyperlipidemia, and Sicca syndrome.

Abbreviations:
AIH, autoimmune hepatitis; ALF, acute liver failure; COBALT, The Clinical Outcomes with OBeticholic Acid in Liver Treatment; IgG, immunoglobulin G; LT, liver transplantation; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; UDCA, ursodeoxycholic acid
It is important to manage them properly to improve the quality of life of the patients. 

PSC is a chronic cholestatic disease of unknown etiology, which is characterized by multifocal stenosis and destruction of the bile ducts owing to inflammation and fibrosis. The prevalence was 4.15 to 16.2 per 100,000 in Northern Europe and North America, while it was 0.95 per 100,000 in Japan. PSC is male-dominant and 50% to 80% of PSC patients in Western countries have ulcerative colitis.

PSC is diagnosed by typical cholangiographic findings and the exclusion of secondary causes. Magnetic resonance cholangiography showed diffuse, multifocal short segment strictures and mild dilatation in the intra- and extrahepatic bile ducts looking like beaded appearance. In contrast to PBC, UDCA did not improve survival, and high-dose UDCA increased the risk of adverse outcomes in patients with PSC. Endoscopic retrograde cholangiopancreatography is indicated to treat the cholangitis with dominant stricture or to take a biopsy when cholangiocarcinoma is suspected. Ultimately, LT should be considered in case of liver failure or decompensated cirrhosis.

In epidemiology, the prevalence and incidence of these rare autoimmune liver diseases presented an increasing trend. However, it is not definite whether this trend reflects a real increase of the disease or improved identification due to better awareness of the physician. In the future, with the effort to find the patients early, it is required to develop more sophisticated treatment approaches individually.

Authors’ contribution
Nae-Yun Heo wrote the manuscript and revised. Haeryoung Kim provided pathologic findings and revised the manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

Instructions for Authors

General Information

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