Aims and Scope

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.

Open Access

The Clinical and Molecular Hepatology is available free in electronic form at www.e-cmh.org. All articles are distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Subscription information

The Clinical and Molecular Hepatology currently offers free online access to all published and ahead-of-print articles. Subscription of the print version is free for the official members of the Korean Association for the Study of the Liver (KASL). If you are a non-KASL member and wish to subscribe the print version of the Clinical and Molecular Hepatology, a subscription fee will be charged annually. To subscribe print version of the Clinical and Molecular Hepatology, please contact the editorial office by e-mail (kasl@kams.or.kr) or by telephone (+82-2-703-0051).

This journal was supported by the Korea Research Foundation of Internal Medicine.

© Copyright 2000-2023 by Korean Association for the Study of the Liver. All rights reserved.
**Special Reviews**

831 Critical appraisal of metabolic dysfunction-associated steatotic liver disease: Implication of Janus-faced modernity  
Gi-Ae Kim, Joon Ho Moon, and Won Kim

844 Waiting for the changes after the adoption of steatotic liver disease  
Eileen L. Yoon and Dae Won Jun

**Reviews**

851 Hepatitis B core-related antigen: A novel and promising surrogate biomarker to guide anti-hepatitis B virus therapy  
Takako Inoue, Takehisa Watanabe, and Yasuhito Tanaka

869 Safety considerations for withdrawal of nucleos(t)ide analogues in patients with chronic hepatitis B: First, do no harm  
Yao-Chun Hsu, Cheng-Hao Tseng, and Jia-Horng Kao

891 Hepatorenal syndrome: Current concepts and future perspectives  
Chan-Young Jung and Jai Won Chang

909 The evolving role of lenvatinib at the new era of first-line hepatocellular carcinoma treatment  
Landon L. Chan and Stephen L. Chan

924 Evidence-based hyponatremia management in liver disease  
Ji Young Ryu, Seon Ha Baek, and Sejoong Kim

945 Carbon ion radiotherapy in the treatment of hepatocellular carcinoma  
Hwa Kyung Byun, Changhwan Kim, and Jinsil Seong

958 Current evidence and the potential role of proton beam therapy for hepatocellular carcinoma  
Sung Uk Lee and Tae Hyun Kim

**Editorial**

969 From nonalcoholic steatohepatitis, metabolic dysfunction-associated fatty liver disease, to steatotic liver disease: Updates of nomenclature and impact on clinical trials  
Ming-Lun Yeh and Ming-Lung Yu

973 Adding to the confusion in more than just the name  
Jacob George
977 Novel paradigm in the treatment of hepatocellular carcinoma: Anticipating breakthroughs with particle therapy
Sang Min Yoon

980 Lean or non-obese nonalcoholic fatty liver disease patients: Are they really lean?
Eugene Han and Yong-ho Lee

984 Chemoembolization combined with radiofrequency ablation is the best option for the local treatment of early hepatocellular carcinoma?
Hyo-Cheol Kim

Original Articles

987 Association of visceral fat obesity, sarcopenia, and myosteatosis with non-alcoholic fatty liver disease without obesity
Hong-Kyu Kim, Sung-Jin Bae, Min Jung Lee, Eun Hee Kim, Hana Park, Hwi Seung Kim, Yun Kyung Cho, Chang Hee Jung, Woo Je Lee, and Jaewon Choe

1002 Differences in liver and mortality outcomes of non-alcoholic fatty liver disease by race and ethnicity: A longitudinal real-world study
Vy H. Nguyen, Isaac Le, Audrey Ha, Richard Hieu Le, Nicholas Ajit Rouillard, Ashley Fong, Surya Gudapati, Jung Eun Park, Mayumi Maeda, Scott Barnett, Ramsey Cheung, and Mindie H. Nguyen

1013 Loco-regional therapies competing with radiofrequency ablation in potential indications for hepatocellular carcinoma: A network meta-analysis
Ha Il Kim, Jihyun An, Seungbong Han, and Ju Hyun Shim

1029 Prognostic role of computed tomography analysis using deep learning algorithm in patients with chronic hepatitis B viral infection
Jeongin Yoo, Heejin Cho, Dong Ho Lee, Eun Ju Cho, Ijin Joo, and Sun Kyung Jeon

Letter to the Editor

1043 Letter regarding “Evidence-based hyponatremia management in liver disease”
Daphne J. Theodorou, Stavroula J. Theodorou, and Ioannis V. Mitselos

1046 Letter regarding “Risk factors in nonalcoholic fatty liver disease”
Abhijit Pratap, Umesh More, Pradnya Phalak, and Anita Deshmukh
Correspondence

1048 Correspondence on Letter regarding "Evidence-based hyponatremia management in liver disease"
Ji Young Ryu, Seon Ha Baek, and Sejoong Kim

1050 Correspondence on Letter regarding "Risk factors in nonalcoholic fatty liver disease"
Eileen L. Yoon and Dae Won Jun

Snapshot

1052 The role of the hepatic autonomic nervous system
Qiankun Luo, Pan Liu, Yifei Dong, and Tao Qin
Critical appraisal of metabolic dysfunction-associated steatotic liver disease: Implication of Janus-faced modernity

Gi-Ae Kim1,*, Joon Ho Moon2,3,*, and Won Kim3,4

1Divisions of Gastroenterology and Hepatology, Department of Internal Medicine, Kyung Hee University Hospital, College of Medicine, Kyung Hee University, Seoul; 2Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam; 3Department of Internal Medicine, Seoul National University College of Medicine, Seoul; 4Divisions of Gastroenterology and Hepatology, Department of Internal Medicine, SMG-SNU Boramae Medical Center, Seoul, Korea

The existing term non-alcoholic fatty liver disease (NAFLD) has raised substantial concerns due to its inherent disadvantages of using exclusionary diagnostic criteria and the stigmatizing word ‘fatty.’ Three pan-national liver associations set out to explore a new nomenclature to replace both NAFLD and its suggested alternative, metabolic (dysfunction)-associated fatty liver disease (MAFLD). They surveyed if a change in nomenclature and/or definition is favored and which nomenclature best communicates disease characteristics and increases awareness. In lieu of NAFLD/MAFLD, metabolic dysfunction-associated steatotic liver disease (MASLD) has been chosen, and an umbrella term, steatotic liver disease (SLD), encompassing the whole spectrum of liver disease, has been proposed. It has been suggested that cardiometabolic risk factors should be considered when categorizing SLD patients. Furthermore, a new subcategory, MASLD with increased alcohol intake (MetALD), casts light on a neglected group of patients with moderate or more alcohol consumption. The importance of metabolic dysfunction was acknowledged in this new nomenclature, but the precise contribution of metabolic dysfunction and alcohol consumption to the development and progression of SLD remains unclear. Herein, we review hepatologists’ and endocrinologists’ perspectives on the new nomenclature, along with its possible impact on clinical practice. Although it is premature to predict the settlement of the new nomenclature, this review may help build more evidence for a soft landing of it in the future. (Clin Mol Hepatol 2023;29:831-843)

Keywords: Metabolic-associated fatty liver disease; Metabolic dysfunction-associated steatotic liver disease; Nomenclature; Non-alcoholic fatty liver disease; Steatotic liver disease

Corresponding author: Won Kim
Department of Internal Medicine, SMG-SNU Boramae Medical Center, Seoul National University College of Medicine, 20 Boramae-ro 5-gil, Dongjak-gu, Seoul 07061, Korea
Tel: +82-2-870-2233, Fax: +82-2-831-2826, E-mail: drwon1@snu.ac.kr
https://orcid.org/0000-0002-2926-1007

*GA Kim and JH Moon contributed equally to this study and are co-first authors.
INTRODUCTION

The term non-alcoholic fatty liver disease (NAFLD) was first introduced in 1986 and was defined as hepatic steatosis affecting at least 5% of hepatocytes in those who consume little or no alcohol without any secondary causes such as viral hepatitis, relevant medications, and lipodystrophy. Thereafter, it has served as the anchor point for established clinical practice (i.e., diagnosis and treatment) as well as exploratory research seeking a better understanding of the disease and the development of biomarkers and drugs. However, its inherent drawbacks of being exclusionary with the use of ‘non-alcoholic’ and stigmatizing with the use of ‘fatty’ prompted a search for an alternative nomenclature.

In recent years, researchers proposed metabolic dysfunction-associated fatty liver disease (MAFLD) as an alternative nomenclature for NAFLD. MAFLD eliminates exclusionary diagnostic criteria and incorporates metabolic risk factors, making it possible to include patients with concomitant liver disease. Nonetheless, it is critiqued for solely relying on metabolic risk factors and not considering alcohol consumption. Moreover, the oversight of non-alcoholic steatohepatitis (NASH), the progressive form of NAFLD, challenged its widespread application in practice. Consequently, a new nomenclature that overcomes the exclusionary and stigmatizing nature of NAFLD and the limitations of MAFLD neglecting both alcohol consumption and NASH was required. Three large pan-national liver associations, including the American Association for the Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), and the Latin American Association for the Study of the Liver, embarked on a modified Delphi process to find a new nomenclature and its definition. Finally, metabolic dysfunction-associated steatotic liver disease (MASLD) was chosen to replace NAFLD and MAFLD, and steatotic liver disease (SLD) was suggested as an umbrella term. In this new nomenclature, patients with SLD are classified into two separate subcategories depending on the presence and absence of a cardiometabolic risk factor (CMRF), and the subcategory with CMRF is further classified into MASLD and MetALD based on the etiology and alcohol consumption.

Hence, this review aimed to examine the suggested nomenclature and its potential impact on screening, diagnosis, treatment, and future drug development. The critical perspectives of the hepatologists and the endocrinologists will be addressed, casting light on the merits and demerits that might arise from this change. Since the new nomenclature has just been proposed, there is a mixture of hope and anxiety, and now may not be the right time to predict its settlement. In that context, this review will contribute to building more evidence for the adoption of the new nomenclature in the future.

THE NEW NOMENCLATURE

MASLD vs. NAFLD/MAFLD: Difference in the diagnostic criteria

MASLD is defined as hepatic steatosis and one or more of the five CMRFs: i) body mass index (BMI) ≥25 kg/m² (≥23 kg/m² for Asians) or waist circumference >94 cm for males and >80 cm for females or ethnicity adjusted; ii) fasting serum glucose ≥5.6 mmol/L (100 mg/dL) or 2-hour post-load glucose levels ≥7.8 mmol/L (≥140 mg/dL) or glycated haemoglobin ≥5.7% (39 mmol/L) or type 2 diabetes or treatment for type 2 diabetes; iii) blood pressure ≥130/85 mmHg or specific antihypertensive drug treatment; iv) plasma triglycerides ≥1.70 mmol/L (150 mg/dL) or lipid-lowering treatment; and v) plasma high-density lipoprotein (HDL) cholesterol ≤1.0 mmol/L (40 mg/dL) for males and ≤1.3 mmol/L (50 mg/dL) for females or lipid-lowering treatment. Patients with SLD and at least one of the CMRFs are categorized as MASLD when they have no other causes of steatosis (Fig. 1).

NAFLD, on the other hand, is diagnosed when there is hepatic steatosis of ≥5% evident on imaging or histology without concurrent liver diseases such as significant alcohol con-
sumption, use of medications that can cause steatosis, or monogenic hereditary disorders. MAFLD shares the same standard with hepatic steatosis in terms of definition but allows for more liberty in its detection. It can be detected by either imaging techniques, serologic biomarkers, or liver histology. Being overweight/obese, having type 2 diabetes mellitus, or having at least two of the metabolic risk abnormalities is a requirement for MAFLD diagnosis. The most significant difference between NAFLD and MAFLD is the recognition of metabolic risk factors for the disease onset and progression. MAFLD adopts a positive criterion rather than a negative one but fails to incorporate alcohol consumption into its diagnostic criteria.

While searching for a new nomenclature, a general consensus was reached that the term ‘metabolic’ should be included and ‘non-alcoholic’ label should be removed. MAFLD first captures patients who are overweight/obese and have type 2 diabetes mellitus and then applies other metabolic risk factors to those with normal weight and normoglycemia. In contrast, the new nomenclature SLD applies the five CMRFs to all patients for classifying its subcategories, including MASLD, MetALD, cryptogenic SLD, and other specific etiology SLD. NAFLD and MASLD differ because SLD adopts a positive criterion and incorporates metabolic risk factors while striving to retain the existing understanding of NAFLD. Furthermore, the new nomenclature pays more sophisticated attention to alcohol consumption, creating the new subcategory, MetALD (Table 1).

The umbrella term SLD

SLD was suggested as an overarching term encompassing a broad spectrum of causes contributing to hepatic steatosis. It is defined as hepatic steatosis identified by imaging or biopsy regardless of etiology. The SLD patients with one or more of the five CMRFs are further categorized into MASLD or MetALD/other combination etiology (Fig. 1). Those without any CMRFs are further categorized into cryptogenic SLD or SLD with other specific etiology.

**Figure 1.** MASLD diagnostic criteria. This figure was adapted from ‘A multi-society Delphi consensus statement on new fatty liver disease nomenclature’ and was modified in the interest of this review. ALD, alcohol-associated liver disease; CMRF, cardiometabolic risk factor; DILI, drug-induced liver injury; MASLD, metabolic dysfunction-associated steatotic liver disease; SLD, steatotic liver disease.
### Table 1. Comparison between NAFLD/MAFLD and MASLD

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NAFLD</th>
<th>Positive criterion</th>
<th>MAFLD</th>
<th>Positive criterion</th>
<th>MASLD</th>
<th>Positive criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td>Negative criterion</td>
<td>Hepatic steatosis of ≥5% by imaging or histology without concurrent liver diseases such as significant alcohol consumption, use of medication that can cause steatosis, or monogenic hereditary disorders</td>
<td>Hepatic steatosis of ≥5% detected either by imaging techniques, blood biomarkers/scores, or liver histology</td>
<td>Hepatic steatosis of ≥5% as diagnosed by imaging or biopsy with one or more of the five CMRFs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definition</td>
<td>Hepatic steatosis of ≥5% by imaging or histology without concurrent liver diseases such as significant alcohol consumption, use of medication that can cause steatosis, or monogenic hereditary disorders</td>
<td>With one of the following: 1) Being overweight/obese 2) Having type 2 diabetes mellitus 3) Satisfying at least two of the metabolic risk abnormalities with lean/normal weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progressive form</td>
<td>NAFLD includes a homogenous population.</td>
<td>MASH</td>
<td>NAFLD is easier to apply in practice since many biomarkers and drugs have already been under development based on the term NAFLD.</td>
<td>MAFLD incorporates the role of metabolic risk factors.</td>
<td>MASLD breaks free from the stigmatizing use of the word ‘fatty’.</td>
<td></td>
</tr>
<tr>
<td>Features</td>
<td>• The use of ‘nonalcoholic’ can be misleading and does not capture the specific etiology of the disease.</td>
<td>• MAFLD incorporates the role of metabolic risk factors.</td>
<td>• MAFLD improves disease awareness and promotes clinical research.</td>
<td>• MASLD provides clear guidance on the amount of alcohol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• The use of ‘nonalcoholic’ can be misleading and does not capture the specific etiology of the disease.</td>
<td>• MAFLD incorporates the role of metabolic risk factors.</td>
<td>• MAFLD improves disease awareness and promotes clinical research.</td>
<td>• MASLD incorporates the role of metabolic risk factors.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• NAFLD classification is rather too strict about the amount of alcohol.</td>
<td>• MAFLD does not have any NASH surrogates.</td>
<td>• MAFLD has MASH as a NASH surrogate.</td>
<td>• MASLD has a separate category of MetALD for those with higher amounts of alcohol consumption.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• The use of ‘fatty’ is stigmatizing.</td>
<td>• MAFLD does not have any NASH surrogates.</td>
<td>• MAFLD has MASH as a NASH surrogate.</td>
<td>MASLD has not yet been fully validated.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CMRF, cardiometabolic risk factor; MAFLD, metabolic (dysfunction)-associated fatty liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic-associated steatohepatitis; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.
liver injury, and monogenic disease.

The adoption of an umbrella term allows for a more intuitive and precise classification of patients. Patients not recognized in the previous nomenclature can be recognized and categorized with this new term SLD. The once-neglected disease awareness can be enhanced, and treatment strategies for those patients can be pursued with more precision. With the existing disease staging and severity not being altered by the new nomenclature, a more coherent and straightforward explanation of the disease can be expected. The leverage of SLD also allows physicians and healthcare providers to better communicate with patients about their conditions and possible therapeutic actions.

CMRFs

Although concerns over the precise meaning of ‘metabolic’ and the varying understanding of the term had been raised, a near-universal agreement was made that considering ‘metabolic disease or dysfunction’ would promote a better understanding of the disease and increase disease awareness since NAFLD has a strong epidemiological and pathogenic link with metabolic diseases such as obesity, diabetes, and insulin resistance. The five CMRFs were selected to align with already well-established and validated risk factors and share their criteria significantly with those of metabolic syndrome. While metabolic syndrome merely considers waist circumference, the CMRF for obesity considers both BMI and waist circumference. As for type 2 diabetes, the CMRF suggests more specific and detailed standards as its criteria. In the pediatric context, although the five CMRF criteria remain the same, the application varies depending on patients’ age.

The new category of MetALD

A new subcategory, MetALD was suggested for the previously neglected group of patients who consume greater amounts of alcohol (20 to 50 g/day for females and 30 to 60 g/day for males) compared to MASLD. Studies have highlighted the need for creating a distinct category of MetALD from MASLD due to the added pathogenic value of alcohol consumption and the prognostic implications that follow. Although there is no explicit reference to the amount of alcohol consumption to be considered significant, EASL and Korean Association for the Study of the Liver guidelines require the exclusion of daily alcohol consumption of >30 g for men and >20 g for women for the diagnosis of NAFLD. Two acronyms, MetALD and MAASLD, were suggested for this group of patients. MetALD was chosen in the interest of avoiding possible confusion and perception associated with the acronym AASLD.

With the introduction of this separate subcategory of MetALD from MASLD, there is an opportunity to generate new knowledge about patients who have both metabolic and alcohol-related risk factors. A recent study that analyzed the UK Biobank data using this new nomenclature found that MetALD patients are more likely to be males and have higher liver enzymes but lower levels of HDL cholesterol compared to MASLD patients. MetALD as a separate category helps define the natural history better and promotes the development of novel biomarkers and new drugs targeting this selected group of patients. MetALD should not be interpreted as a binary category that separates MASLD and MetALD. Instead, it should be understood as a category with a continuum across MASLD and ALD.

HEPATOLOGIST PERSPECTIVE

Implications for enhanced disease awareness

NAFLD is a treatable and preventable disease only if it is diagnosed promptly. Once it progresses to cirrhosis, it is irreversible, and patients suffer inevitable complications related to NAFLD. However, the terminology “NAFLD” misleads patients to believe that there is little potential harm, and finding the real cause of their suffering is challenging. Studies have reported that more than 95% of subjects with suspected NAFLD were unaware of liver disease, and more than 75% did not realize they were at risk of developing NAFLD. There is a growing concern among non-hepatologists that NAFLD is an important liver disease as it often co-exists in patients with diabetes and metabolic syndrome. Even at the risk of confusing practice and the public with
suggestions from NAFLD to MAFLD and yet another change from NAFLD/MAFLD to MASLD, an appropriate term that can increase disease awareness and accelerate biomarker and drug development is critical. A new nomenclature has been announced after a thorough sharing of opinions among experts and relevant stakeholders, and the introduction of MASLD and its simple criteria is expected to bring many positive changes that unify terminology and contribute to the mitigation of disease progression.

**Implications for clinical practice: diagnosis and treatment**

The suggested new nomenclature, such as SLD and MASLD, is expected to affect clinical practice positively. Unlike MAFLD, which does not consider NASH, the new nomenclature maintains NASH, but under a different term, metabolic-associated steatohepatitis (MASH), limiting the confusion in practice. The consensus process has diligently considered preserving existing data and causing limited hindrances to ongoing trials. An analysis of the LITMUS consortium European demonstrated that there is a 98% overlap between patients with conventional NAFLD and those with the newly suggested MASLD. However, further research is needed to adapt this new nomenclature to a specific group with lean NAFLD or SLD without any metabolic risk factors. As for the prevalence, a recent meta-analysis involving 17 studies revealed that MAFLD has a higher prevalence compared to NAFLD (33.0% vs. 29.1%), and future studies should compare the prevalence of MASLD with that of NAFLD.

With the suggested new nomenclature, the diagnostic process is expected to become easier and more intuitive. The shift from a negative to a positive diagnostic criterion means that the excruciating exclusion process can be avoided and diagnoses can be made based on hepatic steatosis and the presence or absence of CMRF. This straightforward diagnosis method can substantially reduce the burden on clinical practice. With the new category, MetALD, and the inclusion of other etiologies, such as viral hepatitis, the range of patients covered by the term SLD is expected to be expansive.

NAFLD is one of the most common liver diseases, affecting millions of patients worldwide. With the overwhelming prevalence reaching almost 30%, identifying at-risk individuals is pivotal. The risk of disease progression to cirrhosis and hepatocellular carcinoma (HCC) along with cardiovascular disease (CVD) and extrahepatic cancer is particularly high among those with NASH. Identifying at-risk individuals is critical to efficiently treat such a prevalent condition with a heterogeneous nature. Recent studies have demonstrated that MAFLD includes more at-risk individuals than NAFLD, showing it has more metabolic comorbidities, elevated liver enzymes, and higher non-invasive liver fibrosis scores. Under the new definition of MASLD, more at-risk patients are expected to be recognized. Additional efforts should be made to identify patients at high risk for MASH or its progression using non-invasive tests or genetics since the current practice of having an invasive biopsy for diagnosing NASH/MASH is rather burdensing.

Concerning treatment, the new nomenclature can benefit more patients as individuals with hepatic steatosis and concurrent liver pathologies can now be recognized and receive timely treatment. Although the current standard treatment for NAFLD stays in lifestyle modifications and weight loss, several phase 3 trials exploring new medical treatments for NASH are in progress with promising preliminary results. Some treatments are expected to receive Food and Drug Administration (FDA) authorization within a few years, allowing healthcare systems to engage more patients.

**Implications for clinical outcomes**

Studies have reported that the mortality rate associated with MAFLD is higher than that associated with NAFLD. Previous studies conducted in Korea and the United States showed that MAFLD was associated with an increased risk of all-cause mortality even after adjusting for metabolic risk factors, whereas NAFLD was not. As for cardiovascular mortality, a nationwide study conducted in Korea showed that CVD risk in the MAFLD group (hazard ratio [HR] 1.43) was higher compared with the NAFLD group (HR 1.09), although both FLD group (HR 1.56) showed the highest risk. However, metabolically dysregulation features rather than MAFLD may have contributed to this higher mortality.

The change from NAFLD to MASLD may help identify a greater number of individuals with metabolic risk factors, and thus, the risk for CVD seems to be higher in MASLD than in NAFLD. Studies comparing all-cause and CVD mortality by SLD subcategory are warranted to validate the prognostic role of each subtype of SLD in predicting CVD. Given that alcohol consumption worsens fibrosis severity in NAFLD,
stratified analysis by the different amounts of alcohol consumption for each MASLD, MetALD, and ALD will enable the development of fine-tuned strategies depending on the behavior of an individual patient.

Cancers, including HCC, are the second leading cause of mortality in NAFLD. A recent population-based cohort study conducted in Sweden showed that biopsy-proven NAFLD carries an increased cancer risk attributable primarily to HCC, in contrast, the contribution of other extrahepatic cancers was modest. This study highlights the need for personalized HCC surveillance schemes across all stages of NAFLD. Future studies are needed regarding which SLD subtype most strongly associated with HCC development. The phenotype of MASLD-HCC in patients at high risk of SLD progression should be further investigated. It also merits further scrutiny of the MASLD-HCC phenotype that best responds to immunotherapy. As HCC surveillance or screening in at-risk populations of NAFLD is a critical issue, the same is expected to extend to MASLD.

Implications for clinical trials and drug development

Clinical trials for developing new drugs consider the presence or absence of NASH as an important eligible criterion. However, the new term MAFLD has introduced confusion since MAFLD abandoned the term ‘steatohepatitis’ and took a rather fluid approach. Concerns have been raised that MAFLD could derail phase 2b and 3 trials designed following the guidance for NASH drug development. This appears problematic because the endpoint of current drug development is the resolution of NASH with no worsening of liver fibrosis. Instead of eliminating the term ‘steatohepatitis’ as a distinguishing subtype, the new nomenclature proposes MASH as the alternative term for NASH, reducing confusion in clinical practice and trials. The new nomenclature also allowed for further characterization of fibrosis severity combined with MASH (e.g., MASH with F3 fibrosis).

With the new categories of MASLD and MetALD, a broader spectrum of patients under the influence of alcohol can be considered in future clinical trials. The new nomenclature does not conflict with ongoing clinical trials or studies, and some drugs in late-phase development, such as semaglutide and resmetirom, can continue their process with the new nomenclature. The FDA approval decision is not expected to be affected by the new nomenclature.

Challenging issues

The newly suggested nomenclature presents several challenges. First, MetALD proposed as a continuum rather than a clear-cut category may make the development of disease-specific biomarkers or drugs specifically targeting this group of patients difficult. Second, the dynamic changes in metabolic health status and alcohol consumption pattern or amount over time per patient may alter the diagnosis depending on the specific time point and should be considered cautiously. Periodic evaluation and repeated monitoring, which are inevitably necessary, may add burdens to clinical practice.

Other challenges may include that using SLD as an umbrella term may result in heterogeneous prognoses by widely encompassing various subcategories of SLD. Lacking a category for SLD patients with CMRFs with alcohol consumption greater than moderate amounts within the four-group classification system can serve as another challenge. Although the varying approaches make the subcategorization different, the discordance between the five-group classification, including separate ALD, and the four-group classification with ALD incorporated in the “other specific etiology SLD” may confuse the application of the new nomenclature. While the five-group classification has hepatitis C virus in its specific etiology SLD, both classifications failed to provide guidance on where hepatitis B should be included. The lack of a definition or a statement on MASLD-related cirrhosis continues to puzzle some professionals. A statement about where patients with MASLD-related cirrhosis are included would help address this issue.

Special attention should be paid to young MASLD patients without CMRF and those with non-obese or lean patients. A recent meta-analysis showed that the non-obese NAFLD population accounts for approximately 40% of the entire NAFLD population globally, and non-obese and lean NAFLD groups still have substantial long-term liver- and non-liver-related comorbidities. Previous studies using the novel diagnostic approach of combining detailed clinical phenotyping and genomic analysis distinguished two types of lean NAFLD. The new nomenclature is void of guidance on where lean NAFLD patients without CMRF fit in. Nonetheless, it should be noted that the suggested nomenclature can pro-
vide room for adding new subtypes depending on future research findings. When categorizing patients with or without CMRFs, there may be certain contexts that necessitate the use of a separate name or title. The possible incorporation of SLD and its subcategories into the International Classification of Diseases 10th Revision codes should be explored by referencing the guidance made by previous literature on the coding of NAFLD. Future studies that can help guide on how to code MASLD diagnosis based on the coding in electronic health records should follow. The localization of the terms in countries that do not use English as their mother tongue and possible confusion or distortion of their meaning in translation should be considered carefully.

**ENDOCRINOLOGIST PERSPECTIVE**

The introduction of MAFLD in 2020 emphasized the role of metabolic imbalance in the etiology of SLD. This paved the way for endocrinologists to play an active role in SLD treatment by seeking to enhance individual metabolic profiles, which would, in turn, potentially improve SLD and its relevant outcomes. While the pathophysiological link between insulin resistance and SLD is well established, it remains unclear whether improving metabolic dysfunction or CMRFs would help treat SLD or prevent its progression to steatohepatitis and fibrosis. From a therapeutic perspective, sodium-glucose cotransporter 2 (SGLT2) inhibitors, commonly used for type 2 diabetes, have improved the prognosis of chronic kidney disease and heart failure in non-diabetic patients. They may also be considered for the treatment of SLD, which reduces intrahepatic fat content and improves liver stiffness, thus expanding their clinical indications. Additionally, thyroid hormone receptor β agonists and glucagon-like peptide-1 (GLP-1)-based therapies, such as GLP-1 receptor agonists, GLP-1/glucose-dependent insulinotropic polypeptide (GIP), GLP-1/glucagon dual agonists, and GLP-1/GIP/glucagon triple agonists, known for their beneficial effects on metabolism, may help reverse SLD. However, their anti-NASH efficacy should be proven in further late-phase clinical trials. These emerging metabolic backbone therapies highlight the therapeutic role of reduced metabolic burden in SLD.

**Pathogenesis of MASLD**

The release of free fatty acids (FFAs) from adipose tissues play a significant role in the pathogenesis and progression of SLD. Adipose tissue serves as a major reservoir of triglycerides, which are composed of FFAs. Under normal physiological conditions, adipose tissue maintains a balance between the storage and release of FFAs depending on the body’s energy demands. However, in pathological conditions such as obesity and insulin resistance, this balance is disrupted. Adipose tissue becomes resistant to the suppressive effect of insulin on lipolysis, leading to an increased release of FFAs into circulation, especially in portal circulation. Elevated levels of circulating FFAs contribute to the development of SLD.

Excessive uptake and accumulation of FFAs in the liver lead to increased triglyceride synthesis and subsequent hepatic steatosis. FFAs modulate the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), which induce liver inflammation and injury. Further accumulation of FFAs promotes inflammation, oxidative stress, and mitochondrial dysfunction in hepatocytes, thereby contributing to the progression of isolated steatosis to MASH and fibrosis.

**Contribution of CMRFs to the development of SLD**

As mentioned above, the primary pathophysiology of MASLD is insulin resistance along with increased release of FFAs. Therefore, the criteria for metabolic dysfunction in patients with MASLD should reflect this pathophysiological condition. As proposed in the new consensus statement for SLD, the definition of metabolic dysfunction in SLD, which relies on the criteria for metabolic syndromes, such as central obesity, high blood pressure, high fasting glucose, high fasting triglycerides, and low HDL cholesterol, may reveal inherent limitations. Metabolic syndrome was initially established to classify individuals at risk of diabetes and/or CVD but not SLD. Thus, the new classification system may not fully reflect the pathophysiological background of fat accumulation in the liver. In particular, diastolic blood pressure and HDL cholesterol are weakly associated with insulin resistance and hepatic steatosis. Without adequate data regarding the predictability of individual metabolic components contribut-
ing to hepatic steatosis, it is challenging to establish whether hepatic steatosis at an individual level is linked to corresponding cardiometabolic risk factors. Further research is required to obtain more precise insights into the relationship between the components of metabolic dysfunction in SLD and the development of hepatic steatosis.

**Cardiometabolic risk threshold required to diagnose MASLD**

The fundamental difference in the diagnostic criteria between MAFLD and MASLD lies in the minimum number of cardiometabolic risk factors required to define metabolic dysfunction. The diagnosis of MASLD requires at least one cardiometabolic risk factor, while the diagnosis of MAFLD requires two or more risk factors. Consequently, significantly more individuals will be classified as having metabolic dysfunction under MASLD than under MAFLD. Metabolic syndrome is typically defined as the presence of three or more cardiometabolic risk factors. Therefore, it is crucial to determine the cardiometabolic risk threshold (i.e., the minimum number of cardiometabolic risk factors needed) to identify metabolic dysfunction in SLD, as it plays a pivotal role in determining the extent to which metabolic syndrome contributes to SLD.

Based on our findings, over 90% of Koreans with SLD had at least one cardiometabolic risk factor (data not shown). This may lead to potential over-classification of MASLD and MetALD but under-classification of pure ALD, cryptogenic SLD, and SLD with specific etiology. Among young overweight or obese individuals, insulin resistance and hepatic steatosis may often exist even without any cardiometabolic risk factors. These young individuals may be misclassified as having cryptogenic SLD despite the presence of insulin resistance because insulin resistance is excluded from the diagnostic criteria of MASLD or MetALD. Classifying >90% of SLD cases as either MASLD or MetALD may mislead patients and clinical practitioners regarding their understanding of the disease.

**Implications of metabolic dysfunction and glucose-lowering agents in the treatment of MASLD**

When refining the new criteria for MASLD, the clinical implications of this classification system should be carefully considered, particularly concerning therapeutic interventions against SLD and its advanced stages. Since MASLD implies metabolic dysfunction as the primary cause of SLD, we may assume that improving metabolic dysfunction can also reverse MASLD.

In principle, SLD treatment is based on lifestyle modifications, including exercise or calorie restriction, to achieve weight loss and accompanying metabolic improvement. However, most people find it challenging to sustain lifestyle modifications in the real world. Thiazolidinediones and vitamin E have shown efficacy in alleviating SLD. In patients with NASH without diabetes, vitamin E as an antioxidant reportedly improved the histological hallmark of NASH, and both vitamin E and pioglitazone significantly reduced liver enzymes as well as intrahepatic fat content. However, both agents failed to demonstrate antifibrotic efficacy and were not free from safety issues. Other phase 3 agents, including obeticholic acid (farnesoid X receptor agonist), elafibranor (peroxisome proliferator-activated receptor-α/δ agonist), selonsertib (apoptosis signal-regulating kinase 1 inhibitor), and cenicriviroc (C-C motif chemokine receptor 2/5 inhibitor), have undergone testing and have shown improvements in NASH. However, none has been approved by the FDA and European Medicines Agency.

SGLT2 inhibitors and GLP-1 receptor agonists are glucose-lowering agents that have the advantage of lowering body weight, which is not easily achieved by lifestyle interventions alone and has been demonstrated to reduce the risk of CVD, the primary cause of mortality in individuals with SLD. As the liver does not express SGLT2 or GLP-1 receptors, improvement of SLD, if any, may be attributed to the indirect effects of these agents, which include weight loss and anti-inflammatory action. A recent review discussed the beneficial effects and potential mechanisms of SGLT2 inhibitors and GLP-1 receptor agonists in treating and preventing SLD. Studies on SGLT2 inhibitors have reported a reduction in intrahepatic triglycerides and reductions in plasma glucose, triglycerides, and body weight. A recent meta-analysis showed that SGLT2 inhibitors slightly improved hepatic steatosis and fibrosis by 12.8 dB/m of controlled attenuation parameter and 0.82 kPa of liver stiffness measurement. In terms of hepatic steatosis, the beneficial effects of SGLT2 inhibitors were more prominent in longer-duration users, younger patients, those treated with dapagliflozin, and those with worse fibrosis and steatosis.
Among many GLP-1 receptor agonist trials, the LEAN trial was the first randomized controlled trial that showed a higher resolution rate in patients with biopsy-proven noncirrhotic NASH. Semaglutide also showed a higher resolution rate of NASH with no worsening of liver fibrosis; however, both trials failed to demonstrate an improvement in the fibrosis stage. Results from two recent meta-analyses were in line with these findings, demonstrating that GLP-1 receptor agonists lead to significant improvements in hepatic fat content, liver enzymes, and other metabolic profiles, including hyperglycemia, but not hepatic fibrosis. These studies or lifestyle intervention trials may provide a more practical definition of metabolic dysfunction in SLD. Examining the inclusion criteria of studies that improved SLD could help identify specific cardiometabolic traits that can benefit from such interventions. To illustrate, studies of SGLT2 inhibitors or GLP-1 receptor agonists are primarily based on individuals with type 2 diabetes and/or obesity, suggesting that these two metabolic components will likely be included in diagnosing MASLD. The methods to ameliorate SLD in individuals with hypertension and low HDL cholesterol are yet to be clarified and require further investigation.

Interaction between insulin resistance and alcohol consumption in the development and progression of SLD

The distinction between MASLD, MetALD, and ALD is not always clear. The overlap subtype, MetALD, indicates the complex interplay between metabolic factors and alcohol as one of the factors contributing to SLD. However, the extent of the contribution of each factor to SLD should be further delineated. Among patients diagnosed with MetALD, there may be some individuals for whom MASLD is considered the primary influencing factor. In contrast, ALD may be the main contributing factor for others.

Metabolic dysfunction and equal or more than moderate consumption of alcohol have additive effects on the progression to advanced fibrosis or severe liver disease, including hospitalization and death. For example, those with moderate alcohol consumption have a ~5-fold increased risk of severe liver disease in each stratum of BMI (<25, 25–30, and >30 kg/m²), wherein the risk of severe liver disease increases with higher alcohol consumption in a dose-dependent manner. However, the exact threshold for alcohol consumption that may lead to liver damage remains unclear. Although some studies have proposed protective effects of mild alcohol consumption, others have indicated no safe level of alcohol consumption, especially among individuals with MASLD. Furthermore, the extent of metabolic dysfunction and the amount of alcohol consumption may vary over time among individuals.

The roles of metabolic dysfunction and alcohol consumption in SLD development and progression are complex and multifaceted. Their relative contributions and interactions remain unclear and are likely to be influenced by other genetic and environmental determinants. Metabolic dysfunction or insulin resistance leads to excessive accumulation of fat in hepatocytes. Furthermore, alcohol increases the level of endotoxins, leading to oxidative stress and endoplasmic reticulum (ER) stress responses, contributing to both steatosis and fibrosis. The independent overlapping mechanisms of metabolic dysfunction and alcohol consumption reciprocally interact and cumulatively contribute to hepatic steatosis, steatohepatitis, and fibrosis progression. An integrated understanding of how these two elements influence the disease trajectory would offer insightful perspectives for more accurate diagnosis and prognostic prediction of SLD subtypes. Ultimately, this would help define and identify SLD subtype-specific biomarkers essential for investigating therapeutic targets.

CONCLUSION

It is generally believed that the term NAFLD poorly communicates its potential harm to patients, making it difficult to confront the real cause of their suffering. With an emphasis on metabolic risk factors, MAFLD has been suggested as an alternative to NAFLD; however, its omission of alcohol consumption and NASH has been raised as a significant concern. Even at the risk of confusing practice and the public, a new term SLD and its four subcategories depending on the presence or absence of CMRF were suggested and MASLD was chosen to replace NAFLD. This new nomenclature is affirmative and non-stigmatizing, and is expected to bring about many changes, increasing disease awareness and involving a broader range of patients. The incorporation of NASH under the new term MASH minimizes confusion in ongoing clinical
trials, and the FDA approval decision for promising agents is expected not to be disrupted.

In the context of SLD pathophysiology, insulin resistance and alcohol consumption are significant risk factors contributing to SLD, each with distinct but occasionally overlapping mechanisms of disease progression. An ongoing challenge in hepatology is understanding the complex interplay between metabolic dysfunction and alcohol use in the pathogenesis of SLD. While metabolic interventions may help effectively improve SLD, the potential for over-classification of MASLD due to an overemphasis on metabolic dysfunction must be carefully considered. Similarly, the contribution of less than moderate alcohol consumption to SLD and disease progression necessitates further research. Both insulin resistance and alcohol consumption require careful consideration in terms of their contributions to SLD for the optimal management of patients.

Nietzsche’s theoretical and practical nihilism, which described both the bright and dark sides of modernization, suggests that MASLD may have the same contradictory features as modernization with two faces of Janus—one facing the past and one facing the future. The new SLD nomenclature is more advantageous and reasonable than the existing NAFLD nomenclature. However, it still needs to be supplemented and improved in several ways. Additionally, in the context of the new nomenclature, it is crucial to emphasize the significance of preserving and building upon existing NAFLD research results while developing new biomarkers and drugs to avoid unnecessary waste of research resources. In this context, further research is strongly encouraged to develop new biomarkers and drugs against MASLD and MetALD to ensure patients benefit the most from disease name changes.

Authors’ Contribution

All authors were responsible for drafting and critical revision of the manuscript.

Acknowledgements

This work was supported by grants from the National Research Foundation of Korea (2022R1F1A1076449 to Gi-Ae Kim), (2021R1C1C1009875 and RS-2023-00222910 to Joon Ho Moon), and (2021R1A2C2005820 and 2021M3A9E4021818 to Won Kim) and the Research Program funded by the Korea Centers for Disease Control and Prevention (2022ER090200 to Won Kim).

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

3. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402.


Waiting for the changes after the adoption of steatotic liver disease

Eileen L. Yoon1,2 and Dae Won Jun1,2

1Department of Internal Medicine, Hanyang University College of Medicine, Seoul; 2Hanyang Institute of Bioscience and Biotechnology, Hanyang University, Seoul, Korea

Steatotic liver disease was suggested as an overarching term encompassing various etiologies of hepatic steatosis. Experts from multinational liver societies went through the Delphi process, including four rounds of surveys, and consented to adopt a new nomenclature and definition instead of the conventional nonalcoholic fatty liver disease (NAFLD). This was to improve the understanding of the patients and primary care physicians, with an explanation of the pathophysiology in the name of the disease. Also, it could minimize the stigmatization of patients by using the histological neutral term “steatosis” instead of “fatty”. Herein, we will discuss the changes and continuity between the two nomenclatures, metabolic dysfunction-associated steatotic liver disease (MASLD) and NAFLD, as well as the challenges to MASLD which need to be addressed in future. (Clin Mol Hepatol 2023;29:844-850)

Keywords: Fatty liver; Nonalcoholic fatty liver disease; Cardiometabolic risk factors; Metabolic syndrome; Social stigma

INTRODUCTION

In the 2023 European Association for the Study of the Liver Congress, ‘steatotic liver disease (SLD)’ was suggested as an overarching term encompassing various etiologies of hepatic steatosis. Experts from multinational liver societies went through the Delphi process about the possible changes, candidates for the nomenclature, impact on the routine clinical practice, etc. They proposed a new nomenclature and definition instead of the conventional “nonalcoholic fatty liver disease” (NAFLD). This was to improve the understanding of disease to patients and primary care physicians (PCPs). Also, it could minimize the stigmatization of patients by using the histological neutral term “steatosis” instead of “fatty”.1

SLD is diagnosed either histologically or by imaging. SLD is further divided into two sub-categories: SLD with cardiometabolic risk factors (CMRF) and SLD without CMRF (Fig. 1). The former is named ‘metabolic dysfunction-associated steatotic liver disease (MASLD)’ when no other etiologies coexist. Metabolic dysfunction is defined as having one or more CMRF, including a body mass index of ≥25; waist circumference of ≥94 cm in Western men and ≥80 cm in Western women; presence of impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus; high blood pressure, high plasma triglyceride levels; lower plasma high-density lipoprotein levels; or dyslipidemia (Table 1). As the consensus was proposed from the Western societies, the cut-offs for waist circumference of Eastern men and women were not clearly provided.

Corresponding author: Dae Won Jun
Department of Internal Medicine, Hanyang Institute of Bioscience and Biotechnology Gastroenterology, Hanyang University College of Medicine, 222-1, Wangsimmni-ro, Seongdong-gu, Seoul 04763, Korea
Tel: +82-2-2290-8338, Fax: +82-2-972-0068, E-mail: noshin@hanyang.ac.kr
https://orcid.org/0000-0002-2875-6139

Editor: Moon Young Kim, Yonsei University Wonju College of Medicine, Korea

Received: Aug. 3, 2023 / Revised: Aug. 30, 2023 / Accepted: Sep. 4, 2023

Copyright © 2023 by Korean Association for the Study of the Liver
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Referring to the cut-offs provided in the metabolic dysfunction-associated fatty liver disease (MAFLD) consensus from the Asian Pacific Association for the Study of the Liver, the cut-offs would be ≥90 cm in Asian men and ≥80 cm in Asian women.

Herein, we will discuss the changes and continuity between the two nomenclatures, MASLD and NAFLD, as well as the challenges to MASLD which need to be addressed in future.

WHAT WOULD CHANGE?

SLD offers a more holistic approach to the management of patients with dual etiology-associated liver disease

In NAFLD, the exclusion of various factors for hepatic steatosis (i.e., alcohol, viruses, and drugs) is the initial major step in diagnosis. Patients with viral hepatitis and hepatic steatosis with nonalcoholic or metabolic causes are diagnosed with viral hepatitis plus fatty liver but not NAFLD. Owing to the absence of approved drugs for NAFLD and difficulty in lifestyle modifications, the presence of fatty liver has been often

**Figure 1.** The classification of Steatotic liver disease. Steatotic liver disease is diagnosed based on the presence of hepatic steatosis identified by imaging or liver biopsy. Metabolic dysfunction-associated steatotic liver disease or metabolic dysfunction-associated alcohol-related liver disease or other combination etiologies are diagnosed with the presence of cardiometabolic risk factors. Steatosis without cardiometabolic risk factors is further sub-classified into SLD with other specific etiology of hepatic steatosis or cryptogenic steatotic liver disease. Figure is modified from the recent consensus proposal. MetALD, metabolic dysfunction-associated alcohol-related liver disease; SLD, steatotic liver disease.

**Abbreviations:**
ALD, alcohol-related liver disease; CMRF, cardiometabolic risk factor; MAFLD, metabolic dysfunction-associated fatty liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction-associated, alcohol-related liver disease; NAFLD, nonalcoholic fatty liver disease; NIT, non-invasive test; PCP, primary care physicians

overlooked.\textsuperscript{3} The impact of hepatic steatosis on the long-term outcomes of viral hepatitis is complicated. For example, hepatic steatosis may lead to a higher chance of hepatitis B surface antigen seroclearance.\textsuperscript{3} However, concurrent hepatic steatosis may exacerbate liver fibrosis,\textsuperscript{4} increase the risk of hepatocellular carcinoma (HCC) and is associated with an increased overall mortality.\textsuperscript{5} Additionally, an increased number of CMRFs is associated with an increased risk of overall mortality, HCC, and extraphepatic cancers.\textsuperscript{6} Therefore, increasing the awareness of both the patients and PCPs for cardiometabolic risks, which may coexist with other liver diseases, is crucial. In this regard, the new term MASLD has enabled us to characterize the multiple etiologies of liver disease and to treat patients holistically by ruling in metabolic dysfunction as the underlying pathophysiology without ruling out alcohol. This allows patients to identify and manage health issues across multiple dimensions. In the presence of other etiologies of steatosis, including significant alcohol intake, it is subclassified as SLD with other etiologies. Alcohol is a major combination etiology of SLD, in addition to CMRFs. Significant alcohol intake was quantified as a weekly intake of 140 g or more in women and 210 g or more in men. Therefore, they named it metabolic dysfunction-associated steatotic liver disease with greater alcohol consumption (MetALD) as the representative of this subclass, which signifies the presence of CMRFs and significant alcohol intake as the dual etiologies of hepatic steatosis. However, this subclass is not confined to MetALD; other combination etiologies of steatosis with CMRFs can be included (Fig. 1).

Overall, the adoption of the overarching term, SLD, offers the advantages of comprehensive patient management and a more holistic understanding of liver diseases.

**New nomenclature provides an increased awareness of the pathophysiology and treatment direction for patients**

It is difficult for patients to understand the pathophysiology and treatment options for NAFLD, as the name only implies that it is not related to alcohol intake. However, with the introduction of the new concepts of MASLD, MetALD, and alcohol-related liver disease (ALD), it could deliver more intuitive messages to patients and PCPs about disease etiology and the direction of future treatment. In particular, MASLD emphasizes metabolic dysfunction as its pathogenesis and conveys a direct message to patients that metabolic parameters should be managed for effective treatment of the disease. In summary, the positive criteria for the diagnosis of MASLD provide patients and PCPs with a more patient-friendly and comprehensible way of communicating about the disease.

**New nomenclature can avoid stigma of ‘fatty’ and ‘alcohol’**

In English-speaking countries, the term “fatty” is often associated with negative connotations, leading to social stigma and patient discomfort. In one study that surveyed 144 patients with NAFLD in a liver clinic located in Spain, 69% of them responded that stigma was perceived to affect all four domains: stereotypes, discrimination, shame, and social isolation.\textsuperscript{8} In another global survey of patients and healthcare providers, 25–31% of the patients felt uncomfortable about the diagnostic terms of NAFLD, while 32–49% of the healthcare providers felt the term was stigmatizing to the patients. Patients would suffer negative stereotypes from the word “Fatty” in that they are perceived to be lazy, unmotivated, and lacking in their willpower to control their self-inflicted disease.\textsuperscript{9}

**Table 1. The criteria for cardiometabolic risk factors for adults**

\begin{tabular}{|l|}
\hline
Body mass index $\geq 25$ kg/m$^2$ (23 kg/m$^2$ for Asians) or waist circumference $\geq 94$ cm (for men); $80$ cm (for women) or ethnicity adjusted Fasting serum glucose $\geq 100$ mg/dL or 2-hour post-load glucose $\geq 140$ mg/dL or HbA1C $\geq 5.7\%$ or type 2 diabetes Blood pressure $\geq 130/85$ mmHg or specific antihypertensive treatment Plasma triglycerides $\geq 150$ mg/dL or lipid lowering treatment Plasma high-density lipoproteins-cholesterol $\leq 40$ mg/dL or lipid lowering treatment \\
\hline
\end{tabular}
To address this issue and prevent unintended social stigma, the new nomenclature task force team has recommended using the histologic term “steatosis,” instead of “fatty” in the diagnosis. A Delphi process was conducted with 236 liver disease professional panelists from 56 countries. The results revealed that 61% and 66% of respondents considered the terms “non-alcohol” and “fatty” to be stigmatizing. Furthermore, 74% of the respondents believed that these terms were significantly flawed and advocated for renaming the condition. On the other hand, the perceptions of stigma from the providers to the patients may differ according to different languages and cultures, for example, 32% from East Asia vs. 49% in the United States. In some regions, there might be no social stigma associated with the terms “fatty” or “non-alcoholic,” and patients may feel comfortable with these labels. Additionally, there could be areas where it is challenging to effectively differentiate between the term “fatty” and the newly proposed term “steatosis” due to unique language characteristics. Depending on the regions and cultural contexts, the transition from “fatty” to “steatosis” may either be ambiguous or not possible. Additionally, there is an issue of over-medicalizing the term as steatosis is confusing to patients. However, this trial aimed to foster a more supportive and understandable environment for patients by minimizing stigmatization, encouraging better communication and care in managing the disease.

The new nomenclature offers coexistence of alcohol use and metabolic risk factors, recognizing a disease spectrum

In real-world scenarios, distinguishing between MASLD and MetALD is intricate and challenging due to the complex interplay of alcohol consumption and metabolic risk factors. Moreover, the classification of MASLD, MetALD, and ALD often faces inaccuracies, as it relies on self-reported alcohol intake data, which can underestimate the actual alcohol consumption in many cases. Despite these challenges, alcohol and metabolic risk factors collectively contribute to an escalated risk of severe liver disease. While cardiovascular disease emerges as the leading cause of death in MASLD, leading cause of death of MetALD and ALD is liver-related mortality. The new nomenclature of SLD offers coexistence of alcohol use and metabolic risk factors, recognizing the conditions as part of a disease spectrum rather than exclusive entities.

MetALD is a category with a continuum across MASLD and ALD depending on the amount of alcohol consumption.

**WHAT REMAINS UNCHANGED?**

MASLD can take over data of epidemiology, non-invasive tests (NITs), and clinical trials from the previous NAFLD era

During the transition of the new nomenclature from the previous NAFLD, several sensitive issues should be addressed. One critical concern is whether previous epidemiologic data and diagnostic cut-offs of NITs can still be applied under the new diagnostic criteria. Additionally, the impact of this new nomenclature on ongoing clinical trials for NAFLD and related drug development is of significant importance. Radical changes in the diagnostic criteria may lead to the loss of valuable epidemiological data that have accumulated over the decades. It will also necessitate the collection of new epidemiological data as well as data on the disease’s clinical course and long-term outcomes under the new nomenclature. This can further necessitate the re-evaluation of the diagnostic performance of various NITs, and the cost-effectiveness of screening strategies based on new disease transition rates. To address these concerns, the nomenclature task force team analyzed data from the LITMUS cohort. Notably, 98% of the NAFLD cohort fulfill the new criteria for MASLD. And very recently proportion of overlap between NAFLD and MASLD was somewhat different from Asian community cohort in Hongkong (97.7%). This suggests that MASLD can take over data of epidemiology, NITs, and clinical trials from the previous NAFLD era.

Alcohol remains a significant risk factor that elevates the likelihood of developing severe liver disease

The new SLD classification underscores the significance of alcohol consumption by categorizing it into MASLD, MetALD, and ALD based on amount of alcohol intake. Numerous research studies have highlighted the correlation between alcohol consumption and heightened risks of liver fibrosis and HCC among individuals with NAFLD, with the extent of risk associated with the quantity of alcohol consumed. This clas-
sification system places ongoing emphasis on both alcohol intake and the management of metabolic factors, by delineating the disease spectrum according to alcohol intake levels.

**FURTHER RESEARCH IN THE FUTURE**

Diagnostic performance of NITs and its cut-offs in MetALD and ALD

SLD is an umbrella term encompassing various conditions, including MASLD, MetALD, ALD, single-specific etiology SLD, and cryptogenic SLD. MASLD is expected to overlap with previous NAFLD patients in approximately 97–98%. This suggests that the NIT and its cut-off applied in previous NAFLD patients can be used without significant changes. However, it is unclear whether previous NITs can be effectively applied in MetALD. Moreover, given the poor diagnostic performance of NITs in patients with alcoholic liver disease, there is a need to develop new NITs tailored to ALD. Each SLD subgroup exhibits distinct characteristics and varying degrees of advanced liver fibrosis, which may necessitate different NITs and their respective cutoff values for an accurate diagnosis. Therefore, validating the diagnostic performance of NITs in SLD subgroups and developing individualized screening algorithms for high-risk groups are needed in these subcategories.

Impacts of potential disease modifiers of SLD, including genetic risk variants, dysbiosis, sarcopenia, and diet

In the recent proposal of the MASLD consensus, SLD without CMRFs is further classified by the criteria of either the presence or absence of a specific etiology of hepatic steatosis: SLD with other specific etiologies or cryptogenic SLD (i.e., SLD without other specific etiologies of hepatic steatosis). Indeed, metabolic dysfunction and alcohol consumption are the major causes of SLD. However, intestinal dysbiosis, genetic variants (e.g., PNPLA3, TM6SF2, etc.), and sarcopenia may also contribute to the development of SLD, especially in lean or non-obese individuals. In the current SLD classification system, lean or non-obese NAFLD may be divided into MASLD and cryptogenic SLD according to the presence of CMRFs, regardless of the genetic variants, intestinal dysbiosis, and sarcopenia. Overemphasis on the CMRFs may overlook or underestimate the involvement of these various disease modifiers, similar to the drawbacks originating from the rule-out diagnosis of NAFLD. In addition, nutrition associated SLD should not be overlooked and it would be further categorized in the cryptogenic SLD. This would also lead to a comprehensive understanding of the contributing factors and improve patient management strategies.

**MAFLD AND MASLD**

MAFLD critically addressed various issues relating negative diagnostic criteria and provided valuable insights for NAFLD. Moreover, MAFLD introduced a concept that offers a more intuitive and fundamental approach by considering the social stigma caused by nomenclature. However, including patients with significant alcoholic liver disease and viral hepatitis in the MAFLD group resulted in increased heterogeneity of the target population based on clinical characteristics and prognosis. This heterogeneity can pose a significant limitation in the development of future disease screening strategies and drug development approaches. In contrast, MASLD effectively resolves the heterogeneity concerns raised by MAFLD, particularly those related to the inclusion of patients with significant alcoholic liver disease and viral hepatitis, while acknowledging the fundamental problems highlighted by MAFLD. Furthermore, the newly introduced MASLD classification encompasses almost the entire previous NAFLD patient population (>95%), enabling a seamless continuation of ongoing clinical trials for NAFLD drugs and successful incorporation of diagnostic tool data. In conclusion, the newly proposed MASLD effectively subdivides the SLD subgroups based on various etiological causes while embracing the important foundational issues presented by the previous MAFLD. This approach maximized the homogeneity of each subgroup, offering a promising pathway for targeted disease screening strategies and drug development.

**NEXT JOURNEY TO SETTLE DOWN OF MASLD**

Indeed, the transition to a new nomenclature for the SLD requires a step-by-step approach to ensure its successful im-
plementation. First, we need more discussions with various stakeholders, including researchers, authorities, and patient groups, regarding clinical trials. We also need to cooperate with liver societies and organizations that have leadership in the Asia-Pacific region. In particular, the Asian Pacific Association for the Study of the Liver officially still supports MAFLD and casts doubt on MASLD. Second, strategies are needed to enhance patient awareness and educate PCPs by disseminating educational materials. It is expected that most patients with SLD will not be managed by hepatologists or gastroenterologists in a referral center, but rather by PCPs. A well-coordinated and comprehensive approach involving all relevant stakeholders will facilitate a successful transition to a new nomenclature for SLD and improve the diagnosis, management, and outcomes of patients with this complex liver disease.

Authors’ Contribution
YEL, first drafting and major revision; DWJ, Supervision and major revision.

Acknowledgements
This work was partly supported by the Korea Drug Development Fund funded by the Ministry of Science and ICT, Ministry of Trade, Industry, and Energy, and Ministry of Health and Welfare HN21C1149000023, Republic of Korea.

This research was supported by a grant of Patient-Centered Clinical Research Coordinating Center (PACEN) funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HC23C0058).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
3. Yoon EL, Jun DW. Changing the nomenclature from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease is more than a change in terminology. Clin Mol Hepatol 2023;29:371-373.
Hepatitis B core-related antigen: A novel and promising surrogate biomarker to guide anti-hepatitis B virus therapy

Takako Inoue¹, Takehisa Watanabe², and Yasuhito Tanaka²,³

¹Department of Clinical Laboratory Medicine, Nagoya City University Hospital, Nagoya; ²Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Kumamoto; ³Department of Virology & Liver unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

The current requirement for biomarkers to detect hepatitis B virus (HBV) infection is polarized. One is a fully-automated and highly sensitive measurement system; the other is a simple system for point-of-care testing (POCT) in resource-limited areas. Hepatitis B core-related antigen (HBcrAg) reflects intrahepatic covalently closed circular DNA and serum HBV DNA. Even in patients with undetectable serum HBV DNA or HBsAg loss, HBcrAg may remain detectable. Decreased HBcrAg levels are associated with reduction of the occurrence of hepatocellular carcinoma (HCC) in chronic hepatitis B. Recently, a fully-automated, novel high-sensitivity HBcrAg assay (iTACT-HBcrAg, cut-off value: 2.1 logI U/mL) has been developed. This attractive assay has been released in Japan very recently. iTACT-HBcrAg can be useful for monitoring HBV reactivation and prediction of HCC occurrence, as an alternative to HBV DNA. Moreover, monitoring HBcrAg may be suitable for determining the therapeutic effectiveness of approved drugs and novel drugs under development. Presently, international guidelines recommend anti-HBV prophylaxis for pregnant women with high viral loads to prevent mother-to-child transmission of HBV. However, >95% of HBV-infected individuals live in countries where HBV DNA quantification is not available. Worldwide elimination of HBV needs the scaling-up of examination and medication services in resource-limited areas. Based on this situation, a rapid and easy HBcrAg assay as a POCT is valuable. This review provides the latest information regarding the clinical use of a new surrogate marker, HBcrAg, in HBV management, based on iTACT-HBcrAg or POCT, and introduces novel agents targeting HBV RNA/protein. (Clin Mol Hepatol 2023;29:851-868)

Keywords: Hepatitis B core-related antigen (HBcrAg); Covalently closed circular DNA (cccDNA); HBV reactivation; Point-of-care testing; RNA destabilizer

INTRODUCTION

Unfortunately, because hepatitis B virus (HBV) relaxed circular DNA is continually converted to covalently closed circular DNA (cccDNA) in the nuclei of hepatocytes,¹² a life-long commitment to chronic hepatitis B (CHB) treatment is required. The current therapeutic goal is to achieve a functional cure, which is characterized by sustained undetectable HBV DNA with the seroclearance of hepatitis B surface antigen (HBsAg). In CHB, it is essential to maintain suppression of HBV replication, which helps to reduce the occurrence of liver-to-liver transmission, liver failure, and hepatocellular carcinoma (HCC). Presently, the mainstay of treatment includes interferon-a and nucleos(t)ide analogs that inhibit HBV polymerase activity. However, the efficacy of these treatments is limited, and the development of resistance is common. Thus, novel agents targeting HBV RNA/protein have been deve-
opened to confirm a functional cure of HBV infection.\textsuperscript{5-8}

Determining the amount and transcriptional activity of cccDNA is also essential for evaluating disease progression and the clinical outcomes of CHB patients.\textsuperscript{9} Hepatitis B core-related antigen (HBcrAg) is a surrogate marker of intrahepatic HBV replication. It has shown a good correlation with conventional HBV biomarkers, including HBV DNA and HBsAg.\textsuperscript{9,11} HBcrAg is a sensitive biomarker of the continued transcription of cccDNA in hepatitis B e antigen (HBeAg)-negative patients, despite marked HBV DNA suppression by treatment with nucleos(t)ide analogues (NAs).\textsuperscript{12} In addition, monitoring HBcrAg may help determine therapeutic effectiveness, as many new prospective therapeutic anti-HBV agents are premised on concomitant use of NAs.\textsuperscript{9}

Currently, the majority of new HBV infections occur in highly endemic areas such as China, Southeast Asia, and sub-Saharan Africa.\textsuperscript{13} The World Health Organization (WHO) has just published guidance, including options for the validation of elimination of hepatitis B and C as a public health problem.\textsuperscript{14} Anti-HBV prophylaxis is recommended for pregnant women with high viral loads to prevent mother-to-child transmission of HBV (DNA ≥200,000 IU/mL).\textsuperscript{15-17} Four options for country-specific aims are set out in the guidance, including the elimination of mother-to-child transmission of HBV, which is discussed in “The global guidance from the WHO for country validation of hepatitis B and C elimination”.\textsuperscript{14} Despite the fundamental role of HBV DNA in the management of CHB, >95% of HBV-infected people live in countries where HBV DNA quantification is not readily available.\textsuperscript{18} Worldwide HBV elimination needs the scaling-up of examination and medication services in low-income and middle-income countries.\textsuperscript{18} Based on these circumstances, a rapid and simple HBcrAg assay is effective for point of care testing (POCT) in regions with limited resources.

As mentioned above, the current demand for HBV biomarkers seems to be polarized: an automated and highly sensitive measurement system vs. a rapid and easy system which can be used for POCT in areas with limited resources. This review describes the clinical use of a new surrogate marker, HBcrAg, in the treatment of CHB or HBV reactivation based on a high-sensitivity HBcrAg assay (iTACT-HBcrAg) and a novel strategy of HBV prevention based on POCT. We also introduce novel anti-HBV agents targeting HBV RNA/protein.

**HBcrAg: A SURROGATE MARKER OF INTRAHEPATIC HBV REPLICATION**

Japan was the first country to recommend testing for HBcrAg in clinical guidelines for CHB management, followed by the greater Asian region and then Europe.\textsuperscript{16,19,20} In this section, we introduce the unique features of the HBcrAg assay and focus on its use in the management of CHB, which includes the ability to predict hepatocellular carcinoma (HCC) (both occurrence and recurrence) and HBV reactivation.

**Components of HBcrAg and the development of its assays**

In 2002, HBcrAg was first reported as a target in the development of a sensitive enzyme immunoassay specific for hepatitis B core antigen (HBcAg) and HBeAg.\textsuperscript{21} Further progress was achieved with the development of an assay for the detection of HBcrAg. HBcrAg comprises three products encoded by the precore/core gene on the HBV genome. HBeAg is a circulating peptide, derived from the precore protein by proteolysis and secreted from the hepatocytes.\textsuperscript{22} HBcAg is a component of the virion and forms the inner nucleocapsid that surrounds the viral DNA. Empty enveloped particles, which contain the 22-kDa precore protein (p22cr/PreC) and phosphorylated HBcAg (pHBcAg), are DNA- and HBcAg-deficient virion-like particles.\textsuperscript{23,24} All three proteins are derived from an identical 149 amino acid sequence.\textsuperscript{23,25} Now, HBcAg, empty particles, and HBeAg can all be measured as HBcrAg by serological testing.\textsuperscript{26,27} The replication cycle of HBV and components of HBcrAg are shown as Figure 1.

**Abbreviations:**

HBV, hepatitis B virus; POCT, point-of-care testing; HBcAg, hepatitis B core-related antigen; HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; NAs, nucleos(t)ide analogues; HBcAg, hepatitis B core antigen; pHBcAg, phosphorylated HBcAg; LAM, lamivudine; ALT, alanine aminotransferase; ETV, entecavir; TDF, tenofovir disoproxil fumarate; CLEIA, chemiluminescent enzyme immunoassay; COI, cut-off index; EASL, European Association for the Study of the Liver; CDC, Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; HIV, human immunodeficiency virus; HCV, hepatitis C virus; CAM, capsid assembly modulator; ASO, anti-sense oligonucleotide; DDS, drug delivery system; iTACT-HBcrAg, high-sensitivity HBcrAg assay; PD1, programmed cell death 1, TLR, toll-like receptor; BCP, basal core promoter
The clinical performance of this assay was evaluated in CHB patients and the HBcrAg concentration correlated positively with the HBV DNA concentration ($P<0.001$) over a 100,000-fold range. 26

The role of HBcrAg in recent clinical assessments

HBcrAg has been used to support the monitoring of CHB and the prediction of clinical outcomes. In this section, we describe the role of HBcrAg in recent clinical applications.

Changes of the amount of serum HBcrAg during NA therapy

In CHB, HBcrAg production persists, even during NA therapy. Among patients positive or negative for HBeAg and treated with NA, 98% had undetectable serum HBV DNA, although intrahepatic cccDNA still could be detected in 51%. 1

Similar findings have been described in several reports. 28-31 Additionally, serum HBcrAg levels at baseline and changes during NA therapy may also serve as suitable indicators in CHB patients. 26,32-35 The details are described in published reviews. 9-11,36

HBcrAg for assessing the possibility of NA cessation

Assessments of serum HBcrAg levels seem to be effective
for identifying patients who do not relapse after stopping NA therapy. Elevated levels of HBcAg (median, 4.9 logIU/mL) predicted relapse in patients treated with lamivudine (LAM) and who had undetectable HBV DNA for at least 6 months prior to stopping therapy. In a similar study, the virological relapse rate within one year of cessation of NA treatment was evaluated in 68 HBeAg-negative patients with CHB. Virological relapse occurred in 37 (54.4%) of them and subsequent analyses revealed that age (>40 years) and an end-of-treatment HBcAg level above 3.7 logIU/mL were factors associated with virological relapse. Conversely, an HBcAg level below 3.4 logIU/mL at the time of cessation of LAM therapy was the only independent factor predictive of not relapsing after the cessation of NA therapy. No patients with HBcAg levels <3.0 logIU/mL at the cessation of therapy developed alanine aminotransferase (ALT) flares.

With reference to the first published papers, the Japan Society of Hepatology Guidelines for the Management of HBV Infection set out the criteria for the cessation of NA therapy. The three laboratory criteria for the cessation of NA therapy are: (A) at least two years administration of NAs; (B) undetectable serum HBV DNA (using real-time PCR); (C) negative serum HBeAg at time of NA treatment cessation; and (D) when the above criteria are met, it is possible to predict the risk of relapse from HBsAg and HBcAg levels at the time of cessation of therapy; low serum HBsAg (<80 IU/mL) and HBcAg (<3.0 logIU/mL) could indicate a group at low-risk of relapse.

Regarding entecavir (ETV) and tenofovir disoproxil fumarate (TDF), the HBeAg level at NA cessation is an independent predictor of relapse, in addition to HBsAg, age, ALT levels, and TDF use. Therefore, the measurement of serum HBcAg may provide a better assessment of patients for whom NA cessation is planned.

The CREATE study group examined the predictors of the loss of detectable HBsAg in a worldwide cohort of 1,216 HBeAg-negative patients with undetectable HBV DNA who discontinued long-term NA therapy. The probability of HBsAg loss after NA cessation varied according to patient ethnicity, HBV genotype and end-of-treatment viral antigen levels. Patients with low HBsAg (<100 IU/mL) and/or undetectable HBcAg levels (<2 logIU/mL), particularly those of non-Asian ethnicity or infected with HBV genotype C, appear to be the best candidates for NA cessation. This report shows that HBcAg is suitable in predicting HBsAg loss after NA cessation.

**HBcAg for predicting HCC occurrence and recurrence**

In this subsection, we introduce the relationship between serum HBcAg levels and HCC occurrence or recurrence in patients receiving NA treatment or not (Table 1).

In treatment-naïve CHB patients, HBcAg was superior to HBV DNA as a predictor of HCC. During a median follow-up period of 10.7 years, an HBcAg level >2.9 logIU/mL was independently associated with HCC development in CHB patients without NA treatment. In another report, among treatment-naïve patients with CHB and an intermediate viral load (serum HBV DNA 2,000–19,999 IU/mL), a serum HBcAg level of 4.0 logIU/mL was an independent risk factor for HCC. Similarly, a serum HBcAg level of 10,000 IU/mL (4.0 logIU/mL) stratifies HCC risk in HBeAg-negative patients in the indeterminate phase.

For treatment-experienced patients, NA reduced, but did not eradicate, the risk of HCC occurrence. In treatment-experienced patients, at the time of disappearance of HBV DNA, the cumulative incidence of HCC at 1, 3, and 5 years was 0.0%, 13.6%, and 17.7%, respectively, in patients with serum HBcAg levels ≥3.4 logIU/mL but was 0.0%, 0.0%, and 2.4%, respectively, in patients with serum HBcAg levels <3.4 logIU/mL (P=0.005). In another report, detectable serum HBcAg (≥3.0 logIU/mL) in CHB patients who received NA treatment for at least two years was an independent risk factor for HCC (hazard ratio [HR]=3.53). Additionally, a post-treatment HBcAg >3.89 logIU/mL predicted progression to HCC (odds ratio [OR]=3.53). Especially, regarding non-cirrhotic patients, HBcAg >3.90 logIU/mL predicted HCC (OR=5.95). Moreover, in HBeAg-negative patients after one year from the start of NA treatment, high HBcAg levels (≥4.1 logIU/mL) were independent predictive factors for HCC development. Regarding the long-term effects of NA treatment on HCC progression, high HBcAg levels and basal core promoter mutations were associated with HCC progression, independent of NA therapy. Hosaka et al. showed the associated between the reduction in HBcAg and the risk reduction of HCC incidence during NA treatment. They concluded that patients with persistently high on-treatment HBcAg levels (cut-off values: 4.9 log U/mL for HBeAg-positive cohort and 4.4 logIU/mL for HBeAg-negative cohort) were more likely to develop HCC, despite sustained viral suppression via long-term NA treatment.
Table 1. The relationship between HBcrAg and HCC development and recurrence

<table>
<thead>
<tr>
<th>HBcrAg assay</th>
<th>CHB Patients</th>
<th>HCC Events</th>
<th>Findings</th>
<th>HBcrAg level (logIU/mL) and point</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional HBcrAg</td>
<td>n=1,031</td>
<td>n=78 (development)</td>
<td>Incidence of HCC for treatment-naïve patients</td>
<td>&gt;2.9 logIU/mL during follow-up period</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>n=2,666</td>
<td>n=209 (development)</td>
<td>At high risk for HCC in treatment-naïve patients with HBV genotypes B or C with intermediate viral load (HBV DNA 2,000–19,999 U/mL)</td>
<td>≥4.0 logIU/mL</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>n=3,462</td>
<td>n=177 (development)</td>
<td>Incidence of HCC for HBeAg-negative, treatment-naïve CHB patients</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>n=133</td>
<td>n=13 (development)</td>
<td>Cumulative incidence of HCC at 1, 3, and 5 years</td>
<td>4.0 logIU/mL (10,000 IU/mL)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>n=109</td>
<td>n=36 (development)</td>
<td>Cumulative incidence of HCC at 1, 3, and 5 years in treatment-experienced patients: 0.0%, 13.6%, and 17.7%</td>
<td>≥3.4 logIU/mL</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>n=228</td>
<td>n=76 (development)</td>
<td>Cumulative incidence of HCC at 1, 3, and 5 years in treatment-experienced patients: 0.0%, 0.0%, and 2.4%</td>
<td>&lt;3.4 logIU/mL</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>n=245</td>
<td>n=14 (development)</td>
<td>HCC development during NA treatment</td>
<td>Detectable HBcrAg during NA treatment</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>n=234</td>
<td>n=57 (development)</td>
<td>Long-term effect of NA treatment on HCC progression</td>
<td>Higher HBcrAg levels (≥4.1 logIU/mL)</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>n=1,268</td>
<td>n=113 (development)</td>
<td>Incidence of HCC in patients with CHB following NA therapy</td>
<td>High on-treatment HBcrAg levels at 1 year (HBeAg+ cohort: 4.9 logIU/mL; HBeAg- cohort: 4.4 logIU/mL).</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>n=449</td>
<td>n=14 (development)</td>
<td>Evaluation of HCC occurrence</td>
<td>HBcrAg &gt;3.0 logIU/mL and HBsAg ≥3.0 logIU/mL (cut-off values)</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>n=55</td>
<td>n=21 (recurrence)</td>
<td>HCC recurrence within 2 years</td>
<td>&gt;4.8 logIU/mL at time of HCC diagnosis</td>
<td>54</td>
</tr>
<tr>
<td>iTACT-HBcrAg</td>
<td>n=17</td>
<td>n=5 (development)</td>
<td>In patients with HCC after HBsAg seroclearance</td>
<td>≥2.7 logIU/mL</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>n=180</td>
<td>n=22 (development)</td>
<td>HCC occurrence of HBeAg-negative patients who received ETV for &gt;1 year</td>
<td>≥2.9 logIU/mL at 1 year</td>
<td>66</td>
</tr>
</tbody>
</table>

HBcrAg, hepatitis B core-related antigen; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogue; HBeAg, hepatitis B envelope antigen; BCP, basal core promoter; HBsAg, hepatitis B surface antigen; iTACT-HBcrAg, high-sensitivity HBcrAg assay; ETV, entecavir.
Incidentally, the concomitant presence of HBsAg and HBcAg can be an efficient biomarker for assessing HCC in patients with CHB. When the cut-off values of serum HBsAg and HBcAg were set at 3.0 IU/mL and 3.0 logIU/mL, respectively, patients with a history of HCC were frequently found among those with low serum HBsAg and high serum HBcAg levels.52

Regarding HCC recurrence-free survival rates, these were significantly lower in HCC patients with high intrahepatic cccDNA and serum HBcAg levels than those with low cccDNA/HBcAg levels (P=0.035, P=0.003, respectively).52 Recently, the predictive value of the serum level of HBcAg for HCC recurrence after curative surgical treatment was validated. In a survey of 55 CHB patients, a serum HBcAg level >4.8 logIU/mL at the time of presurgical HCC detection was related to HCC recurrence within two years of surgery (HR=8.96).54

HBV reactivation
There have been few reports on the effectiveness of conventional HBcAg measurements for predicting HBV reactivation. Among HBsAg-negative and anti-HBc-positive patients undergoing high-risk immunosuppressive therapy, including rituximab for allogeneic hematopoietic stem cell transplantation, those who were HBcAg-positive had a significantly higher 2-year HBV reactivation rate than those who were HBcAg-negative (71.8% vs. 31%, P=0.002).55

A comparison between HBcAg and HBV RNA assays
In this section, the features of serum HBV RNA detection, an HBV biomarker with similar applications to HBcAg, are presented briefly. Circulating HBV RNA might be used as a new serum biomarker for HBV infection, treatment and prognosis.56,57 Huang et al.58 examined the correlation between serum HBV RNA and intrahepatic cccDNA levels and found that serum HBV RNA reflected the cccDNA level in HBeAg-positive CHB patients.59 That is, serum HBV RNA levels differ significantly between HBeAg-positive and HBeAg-negative patients.

Meanwhile, serum HBcAg correlates with intrahepatic cccDNA levels better than HBV RNA and HBsAg, regardless of HBeAg status. Chen et al.60 also assessed the correlation of serum HBcAg with HBV RNA and HBsAg, and investigated whether serum HBcAg was more useful as an indicator of intrahepatic cccDNA in HBeAg-positive and HBeAg-negative CHB patients. Serum HBcAg was found to be better correlated with intrahepatic cccDNA than serum HBV RNA and HBsAg, regardless of HBeAg status.60

A HIGH-SENSITIVITY HBcAg ASSAY PUT INTO PRACTICAL USE IN JAPAN

Characteristics of the new high-sensitivity HBcAg assay
We recently developed and described a fully automated high-sensitivity chemiluminescent enzyme immunoassay (CLEIA) for detecting HBcAg.61 This novel, high-sensitivity HBcAg assay (iTACT-HBcAg; Fujirebio, Inc., Tokyo, Japan) is less expensive than serum HBV DNA assays and can measure serum HBcAg levels within 30 minutes. The procedure involved in the iTACT-HBcAg assay is summarized in Figure 2. The sensitivity (2.1 logIU/mL) of the iTACT-HBcAg assay is approximately 10-fold higher than that of a conventional HBcAg assay (2.8 logIU/mL). This attractive assay has been launched in Japan recently and has made its way into clinical practice. In this section, we discuss which components of HBcAg can be detected to contribute to its high sensitivity and the reported clinical use of this assay.

What does iTACT-HBcAg detect in the sera of patients with HBV reactivation?
The first report of the iTACT-HBcAg assay included the early detection of HBcAg using OptiPrep® density gradient centrifugation analysis.61 The analysis of HBV-related antigens in the serum of a patient prior to HBV reactivation suggested that HBcAg detected 133 days before HBV DNA became detectable might be in empty particles. At that time, both serum HBeAg and HBsAg were undetectable by a conventional assay and ultra-high-sensitivity HBsAg immune complex transfer-CLEIA (ICT-CLEIA; Sysmex Corporation, Kobe, Japan),7 respectively. HBcAg in the fractions that corresponded to the empty particles in a serum sample obtained 49 days after HBV reactivation were in enveloped empty particles. At that point, HBV DNA was again undetectable, HBeAg was positive (2.6 cut-off index), and HBsAg was positive (0.0135 IU/mL by ICT-CLEIA). These data suggest that the particles detected 133 days
before HBV reactivation are similar to the enveloped empty particles detected 49 days after HBV reactivation, and not consistent with unenveloped particles with a greater density.\(^61,62\) To test this hypothesis, an even more sensitive HBsAg assay than ICT-CLEIA\(^7\) is needed, in addition to iTACT-HBcrAg.

### HBV reactivation

iTACT-HBcrAg has an advantage for monitoring patients in the early phase of HBV reactivation. The first report regarding iTACT-HBcrAg confirmed that HBcrAg was detectable by iTACT-HBcrAg before and at the time of HBV DNA positivity in 9 and 2 of 13 patients diagnosed as HBV reactivation.\(^31\) Subsequently, several papers have been published that support this initial report and show more comprehensive clinical features of iTACT-HBcrAg.

Recently, the clinical usefulness of iTACT-HBcrAg was determined in patients with resolved HBV infection after NA treatment for HBV reactivation.\(^63\) Twenty-seven patients with HBV reactivation and systemic chemotherapy for hematological malignancies were analyzed retrospectively. Of 25 patients with detectable iTACT-HBcrAg at the initiation of NA treatment, 17 (68%) achieved iTACT-HBcrAg loss. Recurrence of HBV reactivation after NA cessation was not observed in seven of eight patients who achieved iTACT-HBcrAg loss or were seropositive for anti-HBs during follow-up, except for one without anti-HBs after allogeneic transplantation. These results indicated that iTACT-HBcrAg could be a potential surrogate marker for safe NA cessation in patients with resolved HBV infection after HBV reactivation, in addition to diagnosis.
of HBV reactivation in the early phase.\textsuperscript{63}

In another report, the efficacies of measuring iTACT-HBcAg to detect HBV reactivation and to determine the initiation of NA treatment were assessed.\textsuperscript{64} Of 44 patients with HBV reactivation enrolled in the study, 27 had quantifiable serum HBV DNA (≥1.3 logIU/mL), serum HBV DNA being unquantifiable (<1.3 logIU/mL) in the remaining 17 patients. Of the 11 patients with undetectable HBcAg by iTACT-HBcAg at HBV reactivation and/or thereafter, 10 had unquantifiable HBV DNA and none developed HBV reactivation-related hepatitis. Taken together, iTACT-HBcAg is suitable for monitoring HBV reactivation to determine the initiation of NA treatment.\textsuperscript{64}

Prediction of HCC development

iTACT-HBcAg might also be useful for predicting HCC development (Table 1).

Recently, the occurrence of HBcAg ≥2.7 logIU/mL in patients with HCC after HBsAg seroclearance was reported to be significantly higher than the level of HBcAg in patients who did not develop HCC (100% [5/5] vs. 33% [4/12], \(P=0.029\)). In that report, a low but detectable level of HBcAg measured by iTACT-HBcAg potentially predicted HCC development, even if HBsAg seroclearance was achieved according to a conventional assay.\textsuperscript{65}

In a more recent report, a retrospective cohort study of 180 HBeAg-negative patients who received ETV for more than one year was conducted.\textsuperscript{66} During follow-up, 22 patients developed HCC. The baseline HBsAg levels were not associated with HCC development. However, high HBcAg levels at baseline and at year 1 were significantly associated with HCC development (\(P<0.001\)). The adjusted HR for HCC incidence was significantly lower in patients with HBcAg ≤2.9 logIU/mL at year 1 than in those in the high HBcAg group. These results indicated that the iTACT-HBcAg assay predicted the occurrence of HCC during anti-HBV treatment better than the conventional assay.\textsuperscript{66}

Detection of occult HBV infection in CHB patients with HBsAg loss

Wong et al. evaluated the usefulness of iTACT-HBsAg and iTACT-HBcAg assays in 96 CHB patients with HBsAg seroclearance, documented by standard assays.\textsuperscript{67} At 10 years after HBsAg seroclearance, 20.4% and 64.5% of the patients still had detectable HBsAg and HBcAg, respectively, using the iTACT-assays. Cumulatively, 66 (71%) patients had detectable HBsAg and/or HBcAg.

These results showed that the iTACT assays detected a low level of HBsAg and/or HBcAg in >70% of patients, even 10 years after HBsAg seroclearance. In other words, CHB patients with a functional cure may still harbor a low level of HBV protein expression.

Similarly, there is a report that testing for HBcAg can be valuable in HBV-resolved patients who are negative for HBsAg.\textsuperscript{68} In an HBV endemic region, more than 80% of non-hepatitis B/non-hepatitis C-HCC patients were anti-HBc seropositive, and occult HBV infection, a difficult-to-diagnose liver disease, was considered an important risk factor for non-hepatitis B/non-hepatitis C-HCC development. Hsieh et al.\textsuperscript{68} reported the efficacy of using HBcAg to identify a subset of non-hepatitis B/non-hepatitis C individuals with high sufficient risk of HCC in that area.

Contribution of HBcAg in the development of new drugs

HBcAg is associated with cccDNA, and plays a pivotal role in the evaluation of cccDNA during the development of new agents against CHB. On the other hand, most anti-HBV agents currently under development are likely to be used in combination with NA, rendering the HBV DNA assay unsuitable for drug efficacy evaluation. HBcAg, which is unaffected by NA, may be useful to monitor cccDNA activity in hepatocytes during NA treatment. This will be discussed in detail in the later chapter.

CLINICAL APPLICATION OF THE HBcAg ASSAY IN REGIONS WITH LIMITED RESOURCES

HBV DNA quantification assays and CLEIA for HBcAg have limited availability and are expensive in resource-limited areas. In this section, we describe recent reports of the clinical application of a simple and unique HBcAg assay in resource-limited regions such as Africa.
The first global guidance from the WHO for country validation of hepatitis B and C elimination

New WHO Guidance for country validation of viral hepatitis B and C elimination was released during a joint the European Association for the Study of the Liver (EASL)—the Centers for Disease Control and Prevention (CDC)—the European Centre for Disease Prevention and Control (ECDC) and WHO symposium at the EASL International Liver Congress 2021. This new guidance can contribute to reducing both new infections with hepatitis B and C viruses and deaths from liver cirrhosis and cancer, reaching high coverage (>90%) with program interventions.

Countries are encouraged to pursue the elimination of viral hepatitis B and C together; however, they may choose to apply separately for one of four certification options described below: Option A: elimination of mother-to-child transmission of HBV (as part of triple elimination of human immunodeficiency virus [HIV], syphilis and HBV, or HIV/HBV). Option B: hepatitis C virus (HCV) as a public health problem; Option C: HBV as a public health problem (including elimination of mother-to-child HBV transmission); and Option D: elimination of HBV and HCV together as a public health problem.

In this guidance, elimination of mother-to-child transmission is a major focus. To prevent mother-to-child transmission, NA treatment of HBV is recommended for pregnant women with high viral loads (≥200,000 IU/mL). However, >95% of HBV-infected people live in countries where HBV DNA quantification is not easily available. Based on these circumstances, a rapid and easy HBcrAg assay is required in regions with limited resources.

A simple treatment algorithm based on HBcrAg assay

A quantitative serum HBcrAg assay is 5- to 10-fold less expensive than a quantitative serum HBV DNA assay. Identification of Gambian patients with CHB who had indications for anti-HBV treatment was assessed using a new experimental algorithm that did not include a serum HBV DNA assay. A simple treatment algorithm based on an HBcrAg assay alone, without an HBV DNA assay, yielded a large area under the receiver operating characteristic curve (0.91, 95% confidence interval [CI]=0.88–0.95), with a sensitivity of 96.6% and specificity of 85.8%. These results support the concept that the HBcrAg assay might be suitable to replace a serum HBV DNA assay for identifying patients with CHB who need treatment.

Quantification of HBcrAg using dried blood spots

In a recent report, a standardized method to quantify HBcrAg in dried blood spots to identify HBV-infected people with high levels of viremia was developed and assessed. The limit of detection of HBcrAg in dried blood spots in relation to HBV DNA levels was 19,115 IU/mL (4.281 logIU/mL) across the major HBV genotypes (A, B, C, D, and E). A strong linear correlation was confirmed between HBcrAg levels in dried blood spots and HBV DNA levels (r=0.94, P<0.0001) in samples with high viral loads (range: 3.7–7.0 logIU/mL). The tool which uses HBcrAg in dried blood spots shows promise for identifying HBV-infected patients with high levels of viremia who require anti-HBV therapy.

A rapid test for HBcrAg based on immunochromatography

The measurement of HBcrAg requires CLEIA, which remains unavailable in decentralized locations in regions with limited resources. Therefore, a rapid diagnostic test, based on immunochromatography and enabling the detection of HBcrAg (HBcrAg-RDT), was developed and subjected to analytical/clinical validation.

HBcrAg-RDT can detect high HBcrAg levels and high viremia in serum, plasma, or whole blood. The sensitivity and specificity of HBcrAg-RDT to diagnose HBV DNA levels were 72.7% and 91.7% for ≥2,000 IU/mL, 86.7% and 88.7% for ≥20,000 IU/mL, and 91.4% and 86.3% for ≥200,000 IU/mL. The sensitivity of HBcrAg-RDT was comparable to HBcrAg-CLEIA and its performance did not vary across HBV genotypes. Its low production cost (USD <5), simple specimen preparation, no requirement for equipment/cold chains, operating temperature (39°C), and rapid turnaround time (45 minutes) all favor its use in regions with limited resources. The HBcrAg-RDT methodology is shown in Figure 3.
DEVELOPMENT OF ANTI-HBV THERAPY AND HBCrAg

NAs, currently approved for the treatment of CHB, are excellent agents with potent activity against HBV DNA. However, the efficacy of NAs against HBsAg is poor, and the probability of achieving HBsAg clearance, a “functional cure”, is low with current treatment methods alone. Therefore, novel HBV therapies aimed at HBsAg clearance are being developed vigorously.

Capsid assembly modulator (CAM)

CAM, a novel agent in development, inhibits the reverse transcription of pgRNA into HBV DNA by blocking capsid formation, the site of reverse transcription. In a phase I study, JNJ-6379 reduced serum HBV DNA levels to -2.86 logIU/mL after 4 weeks of treatment, with no change in HBsAg levels. Although CAM is expected to be used in combination with NA, it is difficult to evaluate the efficacy of CAM by HBV DNA during combination therapy with NA. On the other hand, HBCrAg, which is barely influenced by NA, is a useful test for determining the efficacy of CAM combined with NA. However, because the efficacy of CAM monotherapy is limited with little reduction of HBsAg and resistant mutations have emerged, the focus of development has shifted to HBV RNA inhibitors.

Development of HBV RNA inhibitors, agents targeting HBV RNA

HBV RNA inhibitors, a class of direct-acting antiviral agents,
have shown good results in suppressing HBsAg and several drugs have entered clinical trials. In addition, a new class of HBV RNA inhibitor is also being developed and is attracting much attention. This chapter outlines the possible mechanisms of action of the HBV RNA inhibitors and the newer therapeutic agents under development and clinical trials.

HBV RNAs, the 3.5 kb messenger RNA (mRNA) that also functions as pregenomic RNA (pgRNA), 2.4 kb and 2.1 kb mRNAs encoding HBsAg, and 0.7 kb mRNA encoding the HBx protein, are transcribed from the HBV genome, present as cccDNA in the nuclei of hepatocytes (Fig. 4). HBV RNA inhibitors include (1) small interfering RNA (siRNA) and (2) antisense oligonucleotide (ASO), which are oligonucleotide therapeutics, and clinical trials are undergoing. In addition, a new drug class, (3) RNA destabilizer, RNA-binding protein inhibitors, is under development.

**siRNA targeting HBV RNA**

siRNA comprise small double-stranded RNA molecules, consisting of 21–23 nucleotides, that act directly on the target RNA, together with the AGO complex, and cause RNA interference (RNAi) to degrade the mRNA and inhibit expression of the target gene. Currently, several siRNA-based HBV therapeutics are under development. Studies using human liver chimeric uPA/SCID mice (PXB mice) showed that siRNA targeting HBV-RNA reduced serum HBsAg levels by ten-fold or more. JNJ-3989 is a drug complexed with N-acetylgalactosamine (GalNAc), a liver-directed drug delivery system (DDS). GalNAc binds to asialoglycoproteins expressed on the surfaces of hepatocytes, and its use as a DDS enables efficient liver uptake of target molecules. In a phase I/II clinical trial in combination with NA, monthly subcutaneous administration of JNJ-3989 resulted in a decrease HBsAg of at least 1 logIU/mL in all patients and reduced HBsAg to below 100 IU/mL in 88% of patients. Furthermore, the average HBsAg reduction of more than 1 log IU/mL was maintained up to 6 months after the end of the study. JNJ-3989 and other siRNAs currently in phase 2 trials, VIR-2218, RG-6346, and AB-729, showed potent HBsAg-lowering activity, with 85–92% of subjects achieving HBsAg reductions of ≥1 logIU/mL and more than 50% of subjects achieving less than 100 IU/mL. In addition, a phase IIb study (Reef-1 Study) of a three-drug combination treatment comprising JNJ-3989, NA, and CAM (JNJ-6379) is currently underway. It is also important to analyze whether combination therapy reduces cccDNA in future trials, and HBcrAg may contribute, as its effect on cccDNA can be evaluated even

http://www.e-cmh.org

https://doi.org/10.3350/cmh.2022.0434
during NA administration.

**Antisense oligonucleotides (ASO)**

ASOs are short single-stranded DNA or RNA molecules, less than 20 nucleotides long, that are complementary to the target sequence. ASO binds to the complementary RNA and forms a DNA/RNA hybrid, followed by rapid degradation via RNase H1.

ASOs designed for anti-HBV therapy bind to HBV RNA, as 8–10 base DNA strands, with modification to resist degradation by nucleases, and cleavage by RNase H1 prevents translation of the viral protein, which cannot be targeted by NAs.

In a phase II study of GSK3228836 (Beprovirsen, an ASO), of which a primary outcome was a loss of HBsAg and HBV DNA for 24 weeks after the end of treatment, HBsAg was significantly reduced by subcutaneous administration twice a week at the start and once a week thereafter. A higher rate of patients with low baseline HBsAg (≤3 log_{10} IU/mL) achieved the primary outcome (Arm 1: 16% vs. 6%) compared to those with high levels (>3 log_{10} IU/mL). A phase II study is also ongoing for GSK 4388067A.

Adverse events in ASO treatment are mostly mild/moderate, including injection site redness at the time of administration, and the safety profile is considered good.

**Drugs targeting HBV RNA-binding proteins**

A new class of drugs is inhibitors of the RNA-binding proteins that are important for RNA regulation. For HBV RNA, inhibitors of non-classical poly(A) polymerases 5 and 7 (PAPDS/7) are under development as anti-HBV therapeutic agents and have been shown to reduce HBsAg in HBV-infected mouse models.

RG-7834 is a novel oral HBV therapeutic agent, belonging to the dihydroquinolinolindone family of compounds (DHQ), and acts as an RNA destabilizer by inhibiting PAPDS/7 to degrade HBV RNA. RG-7834 has been shown to selectively inhibit HBV transcription in HBV-infected PXB mice. Although the development of RG-7834 has been suspended because of undisclosed adverse effects, it has been modified to avoid effects on other organs by combination with a liver-specific delivery system. AB-452 has also been shown to act as an RNA destabilizer targeting PAPDS/7, and to have superior HBsAg-lowering properties *in vitro*.

The PAPDS/7 inhibitor GSK3965193 is currently in phase I and II trials as a single agent and in combination with Bepirovirsen, an ASO. In preclinical studies of GSK3965193 and Bepirovirsen in an HBV-infected mouse model using AAV-HBV, dose studies were conducted with GSK3965193 and Bepirovirsen as single agents and combination therapy with the two agents. The results showed that GSK3965193 administered per oral as a single agent, resulted in a maximum 1 log reduction of HBsAg levels. Bepirovirsen was also administered subcutaneously with a maximum 2 log reduction of HBsAg levels. Furthermore, a 3-log reduction of HBsAg was observed when both drugs were used in combination, exceeding the effect of either as monotherapy.

**DISCUSSION**

Here, we have discussed the future prospects for HBcrAg diagnostics. HBcrAg is a useful novel biomarker for the management of CHB, including HBV reactivation and predicting HCC occurrence. HBcrAg is appropriate for evaluating the amount of intrahepatic cccDNA in CHB patients. As noted in the introduction, when we look at HBV infection on a global scale, there now appear to be two separate demands for HBV marker assays. One is an automated and highly sensitive assay system and the other is a simple assay system that can be used as a POCT in resource-limited areas.

Just recently, iTACT-HBcrAg was launched in Japan and appeared in clinical practice. The advantages of this assay include that it does not require specific skills, it is less expensive than serum HBV DNA assays, and it has a more rapid turnaround time than the conventional HBcrAg assay, with results available within 30 minutes from the automated system, rather than the approximately 7 hours required for serum HBV DNA assays. Additionally, iTACT-HBcrAg should be valuable for creating and assessing the anti-HBV treatments that are currently under development and that target intrahepatic cccDNA or function as fibrolytic agents.

Recently, Wu et al. mentioned the pros and cons of HBcrAg in their review. At this time, we would like to present data that does not give HBcrAg much credit and ponder points for the future use of HBcrAg. The promising surrogate marker should reflect the kinetics of cccDNA. The serum HBcrAg level strongly reflects the amount of cccDNA prior to NA treatment. Wang et al. have reported that the decrease of
HBcrAg correlated with the decline of cccDNA level after 96 weeks’ NAs therapy in HBeAg-positive patients \( r=0.282, P=0.043 \). In their study investigating the ability of HBcrAg to predict HBV relapse in HBeAg-negative patients after cessation of ETV therapy, Huang et al. reported that a baseline HBcrAg of 4 logIU/mL was the optimal cut-off value for predicting HBV relapse. Meanwhile, HBcrAg at the end of treatment was not a significant predictor of virological or clinical relapse after the cessation of ETV. In another report, Seto et al. described the effectiveness of measuring HBV RNA for determining the suitability of treatment cessation. While end-of-treatment serum HBV RNA and off-treatment serial HBV RNA were both independently associated with HBV DNA >2,000 IU/mL, serum HBcrAg measurement is not essential for deciding on ETV cessation in patients with CHB, especially those with low HBsAg levels. Based on these reports, we have to conclude that the serum HBcrAg level can be valuable for treatment-naïve CHB patients; nevertheless, its value might be limited for patients on-treatment. We need to continue to accumulate and examine clinical data regarding HBV biomarkers, including HBcrAg, HBsAg and HBV RNA, to clarify further the situations in which HBcrAg can be used appropriately.

Moreover, the clinical utility of HBcrAg, compared to conventional markers of HBV replication and disease activity, is unclear. Ghany et al. categorized untreated participants according to the phase of CHB, based on HBsAg and HBeAg status and HBV DNA and ALT levels. Associations of a higher HBcrAg level with higher ALT, APRI, and Fibrosis-4 levels were consistent in the HBeAg-negative, but not HBeAg-positive, phases. Despite clear relationships between the HBcrAg level and CHB phases, these markers have limited additional value in differentiating CHB phases because of their strong association with HBV DNA.

Meanwhile, the development of POCT for more convenient and accurate HBV markers was a long-held desire to efficiently identify individuals infected with HBV. An immunochromatography assay for HBcrAg and the system which uses dried blood spots has shown promise for identifying HBV-infected patients with high levels of viremia who require anti-HBV therapy and its use in regions with limited resources is reasonable. In locations like Africa, where the testing site and home are far apart, we must consider the possibility that once a person leaves the testing site or clinic, he/she may never return. In this respect, the HBcrAg-RDT reported recently makes sense and is an excellent technique consistent with local requirements.

HBV RNA inhibitors potently suppress surface protein synthesis and have excellent HBsAg-lowering properties. They may aim to achieve a functional cure by suppressing the production of HBsAg and contributing to the recovery of exhausted host immune responses. In addition, HBV RNA inhibitors target almost all RNAs transcribed from cccDNA and are expected to be superior HBcrAg inhibitors, among all drugs currently in development. On the other hand, the oligonucleotide therapeutics, siRNA and ASO, are inevitably associated with the problems of possible resistance mutations and off-target effects on the host. In addition, the new agents need further studies not only to prove efficacy but also to define safety better. In uncontrolled clinical trials of Bepirovirsen, adverse events occurred frequently; in particular, elevated ALT levels, which were more common in patients without NA than with NA. It may be better to use NA therapy in combination with these new agents.

RNA destabilizers, a new class of anti-HBV agents, target RNA-binding proteins derived from the host and are therefore unlikely to induce resistance mutations. In addition, they are orally administered drugs, which may contribute to good adherence. Notably, AB-452, an RNA destabilizer, made the pgRNA bands almost completely invisible when used in combination with GLS-4, a CAM. The HBV RNA destabilizer, when used in combination with CAM, may work more effectively by inhibiting capsid formation and keeping pgRNAs bare state. In other words, combining an HBV RNA destabilizer with CAM may have a more potent effect and bring about cccDNA and HBcrAg reduction.

Although siRNA, ASO, and RNA destabilizers are promising drugs with excellent therapeutic effects, they are considered to have limitations in monotherapy as HBsAg may rise again after discontinuation of medication. Therefore, in order to achieve a functional cure, it will be important to develop combination therapy with currently approved NAs, and agents under development that inhibit HBV DNA synthesis, such as core assembly modulators, and combined with immune modulators such as PD1 inhibitors and TLR agonists. HBcrAg could be very useful and make a significant contribution to the development of new therapies.
CONCLUSION

In this review, we have described the clinical use of a novel surrogate marker HBcrAg in the management of CHB and new anti-HBV therapies targeting HBV RNA. With further validation with global studies, HBcrAg is expected to become a next-generation biomarker for many characteristics of clinical practice in CHB.

Authors’ contribution
Conceptualization, T.I., T.W. and Y.T.; Writing the Original Draft, T.I. and T.W.; Writing, Review, & Editing, Y.T.

Acknowledgements
Figure 1 in this article will be published in Comprehensive Guide to Hepatitis Advances, 1st edition, Editors: Wai-Kay Seto, Mohammed Eslam, Noninvasive assessments of disease severity: biomarkers, ISBN: 9780323983686, Copyright Elsevier (2023).

Conflicts of Interest
Lecture Fees: Gilead Sciences, Inc and GlaxoSmithKline PLC. (Yasuhito Tanaka).

REFERENCES

25. Hadziyannis E, Laras A. Viral biomarkers in chronic HBsAg negative HBV infection. Genes (Basel) 2018;9:469.
32. Ma H, Yang RF, Li XH, Jin Q, Wei L. HBcrAg identifies patients failing to achieve HBsAg seroconversion treated with pegylated interferon Alfa-2b. Chin Med J (Engl) 2016;129:2212-2219.


59. Liu YY, Liang XS. Progression and status of antiviral monitoring in patients with chronic hepatitis B: From HBsAg to HBV RNA. World J Gastroenterol 2018;10:603-611.


Safety considerations for withdrawal of nucleos(t)ide analogues in patients with chronic hepatitis B: First, do no harm

Yao-Chun Hsu, Cheng-Hao Tseng, and Jia-Horng Kao

1Department of Medical Research, E-Da Hospital, Kaohsiung; 2School of Medicine College of Medicine, I-Shou University, Kaohsiung; 3Department of Internal Medicine, Fu-Jen Catholic University Hospital, New Taipei; 4Institute of Biomedical Informatics, National Yang-Ming University, Taipei; 5Division of Gastroenterology and Hepatology, E-Da Cancer Hospital, Kaohsiung; 6Department of Internal Medicine and Hepatitis Research Center, National Taiwan University Hospital, Taipei; 7Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

Nucleos(t)ide analogues (NA) are widely used to treat hepatitis B virus (HBV) infection, but they cannot eradicate the virus and treatment duration can be lifelong if the endpoint is set at seroclearance of the hepatitis B surface antigen (HBsAg). As an alternative strategy, finite NA therapy without the prerequisite of HBsAg seroclearance has been proposed to allow treatment cessation in patients with sustained undetectable HBV viremia for two to three years. However, reactivation of viral replication almost always follows NA withdrawal. Whereas HBV reactivation might facilitate HBsAg seroclearance in some, it could lead to serious acute flare-ups in a certain proportion of patients. Occurrence and consequences of NA withdrawal flares are complicated with various factors involving the virus, host, and treatment. Accurate risk prediction for severe flares following NA cessation is essential to ensure patient safety. The risks of life-threatening flares in patients who discontinued NA according to the stopping rules of current guidelines or local reimbursement policies have recently been quantitatively estimated in large-scale studies, which also provided empirical evidence to help identify vulnerable patients at risk of devastating outcomes. Moreover, risk predictors were further explored and validated to hopefully aid in patient selection and management. In this narrative review with a focus on patient safety, we summarize and discuss current literature on the incidence of severe flares following NA cessation, risk stratification for candidate selection, rules of posttreatment monitoring, and indications for treatment resumption. We also share our thoughts on the limitations of existing knowledge and suggestions for future research. (Clin Mol Hepatol 2023;29:869-890)

Keywords: Hepatitis B virus infection; Antiviral agents; Hepatitis B surface antigens; Patient safety

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is the leading cause of liver-related morbidity and mortality, and remains a major threat to global public health, affecting 296 million people worldwide.\(^1\) Antiviral treatment using nucleos(t)ide analogues (NA) effectively inhibits viral replication, ameliorates hepatic necroinflammation, attenuates or reverses liver fibrosis, and reduces the risk of hepatocellular carcinoma (HCC).\(^2,4\) Current NA regimens include entecavir, tenofovir

Corresponding author: Jia-Horng Kao
Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine and Hospital, 7 Chung-Shan South Road, Taipei, 10002, Taiwan
Tel: +886-2-23123456-67307, Fax: +886-2-23709820, E-mail: kaojh@ntu.edu.tw
https://orcid.org/0000-0002-4442-7952
disoproxil fumarate (TDF), and tenofovir alafenamide (TAF), all of which possess high genetic barriers to the selection of resistance-associated variants and sustained viral suppression can be achieved in the vast majority of treated patients. Nevertheless, NA fails to eradicate HBV because their pharmacological activities are confined to the reverse transcriptase domain of the viral polymerase, without direct effects on the episomal covalently closed circular HBV DNA (cccDNA) and fragments of viral DNA integrated into the host genome, both of which can be transcriptionally active despite NA treatment. NA withdrawal almost inevitably reactivates viral replication with potential clinical consequences.

The optimal duration of NA treatment for chronic HBV infection has not been clearly defined and recommendations are inconsistent among current guidelines. It is generally acceptable to stop NA after seroclearance of hepatitis B surface antigen (HBsAg), or the so-called functional cure, which signifies quiescence of replicational and transcriptional activities of the virus and predicts durable remission off NA treatment. Unfortunately, HBsAg seroclearance rarely occurs during ongoing NA treatment with an annual incidence rate below 1%. This treatment endpoint is literally synonymous with indefinite treatment duration for most patients receiving NAs.

Indefinite NA treatment may have several theoretical disadvantages, including the challenge of maintaining medication adherence over an extended period, the concern of long-term toxicity from prolonged drug exposure, and the financial burden of sustained prescription costs. More importantly, it was found that the incidence of HBsAg seroclearance was paradoxically higher after NA discontinuation compared to during treatment, and a finite NA strategy of “stop to cure” was thus proposed. The practice of stopping NA therapy without the requirement of HBsAg seroclearance is currently debatable with both perceived pros and cons (Table 1). For patients who have initiated NA therapy with positive hepatitis B e antigen (HBeAg), current guidelines generally agree treatment cessation may be considered after achieving HBeAg seroconversion, followed by consolidation for one year or longer. Contrary to HBsAg seroclearance, however, HBeAg seroconversion does not consistently predict sustained remission after NA cessation, even with additional consolidation for several more years. Such contradictory recommendations highlight the limitations of current knowledge on this issue. Central to the controversy is the concern about patient safety and most concerning is the risk of acute-on-chronic liver failure (ACLF),

Table 1. Presumed pros and cons of the finite nucleos(t)ide analogue therapy for chronic hepatitis B virus infection

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔️ Triggering virus-specific immune reactions to control the infection</td>
<td>· The provoked necroinflammation can be fulminant or unremittent</td>
</tr>
<tr>
<td>✔️ The chance of HBsAg seroclearance is higher compared to continuous therapy</td>
<td>· The risk of acute hepatitis flare is not always predictable and potentially fatal</td>
</tr>
<tr>
<td>✔️ Addressing the difficulty of adherence to long-term medication</td>
<td>· Close follow-up and easy access to healthcare may not be guaranteed</td>
</tr>
<tr>
<td>✔️ Easing the financial burden incurred by drug prescription</td>
<td>· Adding expenses on posttreatment monitoring and managing flares</td>
</tr>
<tr>
<td>✔️ Relieving the concern of uncertain toxicity from prolonged drug use</td>
<td>· Arousing anxiety about the uncertainty of HBV reactivation</td>
</tr>
</tbody>
</table>

The arguments could be theoretical without a firm base of empirical evidence.

Abbreviations:
NA, nucleos(t)ide analogues; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e antigen; ACLF, acute-on-chronic liver failure; APASL, Asian Pacific Association for the Study of the Liver; ALT, alanine aminotransferase; ULN, upper limit of normal; CI, confidence internal; AASLD, American Association for the Study of the Liver; EASL, European Association for the Study of the Liver; EOT, end of therapy; HBcrAg, hepatitis B core-related antigen; JSH, Japan Society of Hepatology; HR, hazard ratio

https://doi.org/10.3350/cmh.2022.0420
Table 2. Current guidelines for the criteria of stopping NA in patients with HBeAg-negative hepatitis B*

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Non-cirrhosis</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International guidelines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AASLD⁹</td>
<td>· Indefinite treatment duration (Quality and Certainty of Evidence: Low Strength of Recommendation: Conditional)</td>
<td>· Treatment discontinuation not recommended</td>
</tr>
<tr>
<td></td>
<td>· May be considered in patients with HBsAg loss, but evidence insufficient</td>
<td></td>
</tr>
<tr>
<td>APASL¹⁰</td>
<td>· HBsAg loss following either anti-HBsAb seroconversion or at least 12 months of a post-HBsAg clearance consolidation period (B1), or</td>
<td>· May be considered with a careful off-therapy monitoring plan (A1)</td>
</tr>
<tr>
<td></td>
<td>· Treatment for ≥2 years with consolidation ≥1 year (B1)</td>
<td></td>
</tr>
<tr>
<td>EASL¹¹</td>
<td>· HBsAg loss, with or without anti-HBsAb seroconversion (Evidence level II-2, grade of recommendation 1), or</td>
<td>· Treatment discontinuation not recommended</td>
</tr>
<tr>
<td></td>
<td>· May be considered after consolidation ≥3 years if close monitoring can be guaranteed (Evidence level II-2, grade of recommendation 2).</td>
<td></td>
</tr>
<tr>
<td>WHO¹²</td>
<td>· Life-long therapy in general</td>
<td>· Treatment discontinuation not recommended</td>
</tr>
<tr>
<td></td>
<td>· Discontinuation may be considered exceptionally in persons who can be followed carefully long term for reactivation and with persistently normal ALT levels and persistently undetectable HBV DNA levels</td>
<td></td>
</tr>
<tr>
<td></td>
<td>· May be considered in persons who have evidence of persistent HBsAg loss and after completion of at least one additional year of treatment, regardless of prior HBeAg status.</td>
<td></td>
</tr>
<tr>
<td><strong>National guidelines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada²⁵</td>
<td>· HBsAg loss (moderate recommendation; class 2, level B)</td>
<td>· HBsAg loss or indefinite duration (moderate recommendation; class 2, level B)</td>
</tr>
<tr>
<td></td>
<td>· HBsAg loss or indefinite duration (moderate recommendation; class 2, level B)</td>
<td></td>
</tr>
<tr>
<td>China²⁶</td>
<td>· The therapy aims are “clinical cure” (i.e., functional cure)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>· No recommended criteria for stopping treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>· No specific recommendations for patients with cirrhosis</td>
<td></td>
</tr>
<tr>
<td>Japan²⁷</td>
<td>· In general, it is necessary not to stop administration of the NAs</td>
<td>· Long-term treatment (Level 5, Grade B)</td>
</tr>
<tr>
<td></td>
<td>· HBsAg loss (can be considered)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>· Treatment for ≥2 years without detectable HBV DNA or high relapse risk score according to serum HBcrAg and HBsAg levels</td>
<td></td>
</tr>
<tr>
<td>Korea²⁸</td>
<td>· HBsAg loss (A1).</td>
<td>· Long-term treatment (B1)</td>
</tr>
<tr>
<td></td>
<td>· With reference to HBsAg level, cessation of NA therapy could be considered (B1).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>· HBcrAg and HBV RNA can be performed when considering cessation of NA therapy (B2)</td>
<td></td>
</tr>
<tr>
<td>Sweden²⁹</td>
<td>· HBsAg loss (B1).</td>
<td>· Long-term treatment (A1)</td>
</tr>
<tr>
<td></td>
<td>· May be considered after long-standing treatment response but require close monitoring after termination (B2)</td>
<td></td>
</tr>
<tr>
<td>Turkey³⁰</td>
<td>· HBsAg loss</td>
<td>· Long-term treatment</td>
</tr>
</tbody>
</table>

AASLD, American Association for the Study of the Liver; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; anti-HBsAb, anti-hepatitis B s-antibody; DNA, deoxyribonucleic acid; EASL, European Association for the Study of the Liver; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B virus s-antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; RNA, ribonucleic acid; WHO, World Health Organization.

*General principle, not for special population such as patient under immunosuppressants or post organ transplantation.
which is potentially fatal.\textsuperscript{31}

Amid interests in and disputes over the finite approach of NA treatment for chronic HBV infection, there is a growing body of evidence reporting clinical outcomes following NA withdrawal.\textsuperscript{32} Knowledge about the safety of treatment cessation is indispensable to inform the practice of finite NA therapy, and data have been emerging to quantify the risk of severe withdrawal hepatitis flares and to identify the associated risk factors.\textsuperscript{33-35} In this review with a special focus on patient safety, we summarized the data available to date and discussed the selection of patients suitable/unsuitable for NA cessation, patterns of off-therapy flares with implications for patient monitoring, and pragmatic considerations for the timing to resume antiviral therapy. We also stressed current knowledge gaps, pointed out pitfalls in existing literature, and provided our perspectives on future directions of research.

\section*{Risk of Serious Acute Events According to Current Stopping Rules}

\subsection*{Incidence of acute hepatitis flares following NA withdrawal}

The risk of acute flare-up, a unique feature of chronic HBV infection that can abruptly develop without preceding symptoms and rapidly progress to liver failure within weeks,\textsuperscript{36,37} must be factored in the consideration of finite NA therapy. It is not surprising to observe acute hepatitis flares following NA withdrawal, because the sudden increase of viral replication along with surge in viremia is known to trigger host immunity against HBV,\textsuperscript{38} as seen in the setting of immunosuppressive treatment without antiviral prophylaxis.\textsuperscript{39,40}

The incidence and “natural history” of HBV flares induced by NA withdrawal can be discerned in recent studies from Taiwan, where the reimbursement coverage for anti-HBV therapy is finite (usually three years of treatment) in principle, and patients need to withhold retreatment according to the strict criteria for treatment eligibility.\textsuperscript{9-11} In a large hospital-based cohort study that applied the Asian Pacific Association for the Study of the Liver (APASL) criteria to select and monitor patients, Liu and colleagues found that acute flares (defined by elevation of serum alanine aminotransferase (ALT) $\geq$5 times the upper limit of normal [ULN]) occurred in 516 out of 1,234 patients (41.8\%) within two years after treatment cessation, and reported that the corresponding cumulative incidence reached 42\%.\textsuperscript{35} In a population-based study with a less selected patient population to represent real-world practice in Taiwan, Hsu and colleagues estimated the cumulative incidence of acute flares (ALT $>$200 U/L) was 30.7\% (95\% confidence interval [CI], 29.4–32.0\%) at four years.\textsuperscript{41}

\subsection*{Risk estimation for severe withdrawal flares with hepatic decompensation}

Most but not all of the withdrawal flares can wane spontaneously or subside with NA retreatment. A severe episode may either present with or progress to ACLF that, in turn, could lead to mortality or the need of liver transplantation. Quantifying the risk of such a severe episode is hence important, but was found to be difficult in earlier studies with small samples and/or few events.\textsuperscript{42} This crucial gap in knowledge has recently been addressed by large cohort studies with more than 500 participants and also by pooled analyses of individual studies (Table 3).\textsuperscript{33-35,43-46} In the Taiwanese population-based study by Hsu and colleagues, the cumulative incidence of severe flares with hepatic decompensation (defined by both hyperbilirubinemia and coagulopathy) was estimated at 1.8\% (95\% CI, 1.5–2.2\%) at four years after treatment withdrawal.\textsuperscript{41} After pooling fifteen studies with 4,525 individual patients in a systematic review and meta-analysis of current literature updated to August 2022, Tseng and colleagues reported that 1.2\% (95\% CI, 0.70–2.1\%) of patients would develop severe flares or hepatic decompensation (variably defined in respective studies) after stopping NA. The international RETRACT-B consortium also found that the cumulative incidence of hepatic decompensation (defined as hyperbilirubinemia, coagulopathy, or clinical complications) was 1.8\% at five years.\textsuperscript{33}

On the basis of these aforementioned studies, life-threatening flares were expected to occur in approximately 1–2\% of HBV-infected patients who discontinued NA according to the stopping rules of earlier practice guidelines (mainly APASL) or local reimbursement policies. Notably, while the criteria of the APASL guidelines are the most stringent for treatment initiation,\textsuperscript{9,11} they are comparatively more liberal than those of the European Association for the Study of the Liver (EASL) and American Association for the Study of the Liver (AASLD) guidelines for stopping NA therapy (Table 2).
Table 3. Summary of large-scale studies (>500 participants) and meta-analyses on the risk of severe withdrawal flares with hepatic decompensation or liver failure

<table>
<thead>
<tr>
<th>Cohort studies</th>
<th>Study type/ Region</th>
<th>Number</th>
<th>Age</th>
<th>Pretreatment status</th>
<th>Criteria</th>
<th>Severe hepatitis flares or decompensation</th>
<th>Severe adverse events</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsu et al. <a href="2021">44</a>*</td>
<td>EHR/Taiwan</td>
<td>665</td>
<td>50.3</td>
<td>14.3%</td>
<td>26.0%</td>
<td>Taiwan reimbursement</td>
<td>Taiwan reimbursement</td>
<td>24 (3.6%)</td>
</tr>
<tr>
<td>Liu et al. <a href="2022">37</a></td>
<td>Prospective/ Taiwan</td>
<td>1,234</td>
<td>56.1</td>
<td>40.1%</td>
<td>0%</td>
<td>Taiwan reimbursement</td>
<td>Taiwan reimbursement</td>
<td>24 (3.6%)</td>
</tr>
<tr>
<td>Hsu et al. <a href="2021">41</a></td>
<td>EHR/Taiwan</td>
<td>10,192</td>
<td>50.9</td>
<td>10.7%</td>
<td>n.a.</td>
<td>Taiwan reimbursement</td>
<td>Taiwan reimbursement</td>
<td>132 (1.3%)</td>
</tr>
<tr>
<td>Ma et al. <a href="2019">44</a></td>
<td>Prospective and retrospective/ Taiwan</td>
<td>535</td>
<td>50.7</td>
<td>0%</td>
<td>29.9%</td>
<td>APASL 2012</td>
<td>Taiwan reimbursement</td>
<td>7 (1.3%)</td>
</tr>
<tr>
<td>Sonneveld et al. <a href="2022">45</a></td>
<td>Prospective and retrospective/ International</td>
<td>572</td>
<td>52.0</td>
<td>n.a.</td>
<td>16.6%</td>
<td>As per institution</td>
<td>As per institution</td>
<td>2 (0.4%)</td>
</tr>
</tbody>
</table>
### Table 3. Continued

<table>
<thead>
<tr>
<th>Cohort studies</th>
<th>Pretreatment status</th>
<th>Criteria</th>
<th>Severe hepatitis flares or decompensation</th>
<th>Severe adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author (year)</td>
<td>Study type/Region</td>
<td>Number</td>
<td>Age</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cirrhosis</td>
<td>HBeAg(+)</td>
<td>Stopping NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Resuming NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe hepatitis flares or decompensation</td>
<td>Death or liver transplantation</td>
<td>Risk factors</td>
</tr>
<tr>
<td>Meta-analysis study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tseng et al. (2022)</td>
<td>50 articles reporting safety outcomes after NA cessation</td>
<td>15 studies (4,525 patients) pooled for risk estimate of overall population</td>
<td>- Heterogeneous design among studies (e.g., stopping rules, retreatment criteria, and definition of decompensation)</td>
<td>- Serious adverse events not often reported in smaller studies with shorter follow-up duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 studies (3,731 patients) pooled for risk estimate of non-cirrhotic population</td>
<td>- Risk estimate (95% CI):</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 studies (744 patients) pooled for risk estimate of cirrhotic population</td>
<td>Overall</td>
<td>Non-cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Severe hepatitis flares or decompensation</td>
<td>1.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Death/Liver</td>
<td>0.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>transplantation</td>
<td>(0.7–2.1%)</td>
</tr>
</tbody>
</table>

For studies from similar institutions, we chose the most representative one such as the larger sample size or more detailed information about the adverse events.

APASL, Asian Pacific Association for the Study of the Liver; CI, confidence interval; EOT, end-of-treatment; EHR, electronic health record; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B s-antigen; n.a., not available; NA, nucleo(s)tid analogue; TDF, tenofovir disoproxil fumarate.

*Data from subgroup of patients exclusively receiving ETV or TDF; †mean, ‡median, §each study with its on definitions of severe hepatitis flares or decompensation, ‡the number from another publication from RETRACT-B cohort.*
Identification of vulnerable patients at excessive risk

Not all patients carry the same risk of withdrawal flares, and those at excessive risk of serious clinical outcomes should be advised against treatment cessation. It stands to reason that older patients and individuals with far advanced diseases are more vulnerable to devastating flares and this reasoning is now supported by empirical evidence. In a hospital-based study that followed the APASL guidelines to stop NA therapy, Jeng and colleagues found all seven patients who developed hepatic decompensation, and three patients who subsequently died, had liver cirrhosis. In larger studies that were statistically powered to explore risk predictors, liver cirrhosis was consistently identified as a risk factor significantly and strongly associated with serious clinical events following NA withdrawal. It has also been shown that an older age was associated with excessive risk of severe withdrawal flares; more specifically, the risk significantly increased with age when the patients were 50 years or older. A higher risk was also observed in patients with a past history of hepatic decompensation or severe acute exacerbation.

Selection of candidates for finite NA therapy

High retreatment rates without accurate patient selection

In addition to vulnerable patients at excessive risk of serious flares, individuals who are prone to relapses of clinical hepatitis and unlikely to clear HBsAg following treatment cessation are not suitable to practice finite NA therapy. Stopping NA in these patients bound to resume treatment only brings about unnecessary and potentially harmful interruption in viral inhibition and possible worsening of liver fibrosis. In fact, about half of the patients who stopped NA according to the rules recommended by earlier guidelines developed active hepatitis that fulfilled the usual criteria for antiviral therapy. Such high retreatment rates reflect the insufficiency of current stopping rules to select candidates for finite NA therapy. On one hand, reactivation of viral replication following NA cessation often induces host immune responses that may facilitate seroclearance of HBsAg. On the other hand, the elicited immune reaction could also result in severe necroinflammation with serious clinical consequences. It is currently unclear how this double-edged sword can be used to clear HBsAg without causing collateral damage. Notably, patients who developed clinical relapse (usually defined as serum ALT >2×ULNs in addition to HBV viremia >2,000 IU/mL) did not have a higher incidence of HBsAg seroclearance than those who remained clinically uneventful. Therefore, the practice of finite NA therapy requires prediction tools that can precisely identify patients at a lowest risk of clinical relapse and also a highest chance of HBsAg loss.

Risk predictors-HBsAg

A number of risk predictors, which can be grouped into virus, treatment, and host factors, have been reported to help distinguish patients at different risks of clinical relapse off NA therapy (Fig. 1). Serum level of HBsAg at the end of therapy (EOT) is the most extensively studied and widely validated among them. It is useful to predict both the risk of hepatitis relapse and opportunity of HBsAg loss. In general, the lower the level of EOT HBsAg, the less risk of clinical relapse, and the higher chance of HBsAg seroclearance. A cutoff point set at 100 IU/mL was proposed and later validated as the easily applicable threshold to select patients who may consider treatment cessation. It should be noted, however, that the risk of clinical flares is lower but not negligible with an EOT HBsAg level <100 IU/mL. The risk can be further decreased by lowering the threshold, but clinical relapse may still occur with an EOT HBsAg level <40 IU/mL, as demonstrated in the study by Tseng and colleagues. Moreover, emerging data indicated that the EOT level of HBsAg did not predict severity of clinical relapse. Fatal flares could still occur in patients with a serum level of EOT HBsAg <10 IU/mL.

Risk predictors-HBcrAg

Quantitation of hepatitis B core-related antigen (HBcrAg) in serum can be used to gauge transcriptional activity of the virus and may add to the accuracy of risk prediction based on HBsAg at EOT. Hsu and colleagues demonstrated that both EOT levels of HBsAg and HBcrAg were both independent risk factors associated with clinical relapse off NA therapy although these two biomarkers were positively correlated with each other. Nevertheless, how to apply HBcrAg on top
of HBsAg in the process of decision making to select candidates for NA cessation remains to be defined. In patients with a low EOT HBsAg (e.g., below 100 or 200 IU/mL), the level of HBcrAg at EOT was found unable to further stratify their relapse risks. On the other hand, encouraging results were reported in studies that simultaneously combined the two biomarkers together instead of placing one after the other in successive steps.

Risk predictors-other viral factors

Other HBV biomarkers that have been reported to predict responses off NA therapy include serum viral load and HBeAg status at treatment initiation, titer of serum HBV RNA at EOT, HBV genotypes, and diversity of viral quasispecies. A lower serum HBV DNA (e.g., <200,000 IU/mL) and negative HBeAg status at the start of antiviral therapy were associated with a lower risk of off-NA relapse. In patients with a low EOT HBsAg (e.g., <200 IU/mL), detectable HBV RNA in serum were found to foretell a higher rate of virological relapse. In addition, HBV genotype C versus genotype B was linked to a greater chance of HBsAg loss in Asian patients although the association with off-NA relapse was inconsistent across studies. A higher pretreatment HBV quasispecies diversity was also found to predict sustained virological response (HBV DNA <2,000 IU/mL) for more than one year off therapy.

Risk predictors-host factors

Host factors that are reportedly associated with relapse or remission off NA include age, genetic polymorphisms, ethnicity, circulatory biomarkers of inflammation or immunity, and HBV-specific immune response. As seen in the association with serious life-threatening flares, an older age has been shown to be a significant risk factor for relapses of clinical hepatitis overall. A pilot study by Su and colleagues found that polymorphisms of CTLA4 and HLA-DPA1, both of which were genes involved in regulation of immune reactions, were predictive of off-NA relapse and clinical response, respectively. There were also preliminary data suggesting that circulatory biomarkers of immunity, such as titers of hepatitis B core antibody (anti-HBc) and RNA levels of IFNγ, IL-8, FASLG, and CCL4 genes from peripheral blood mononuclear cells, could help identify patients who would suffer relapses off NA treatment. In fact, a biomarker of hepatic necroinflammation readily available in routine clinical care, i.e., the serum level of ALT, may also add to the risk pre-
prediction of off NA relapse. The chance of HBsAg seroclearance was also significantly higher in Caucasian patients than in Asian patients although the incidence of ALT flares did not appear to differ by ethnicity. This association might reflect differences in the geographic distribution of HBV genotypes, because the genotype was predominantly A or D in Caucasians, but B or C in Asians. The mode of transmission, the age/duration of infection, or host genetic polymorphism should also be considered. Further research is warranted.

Risk predictors—HBV-specific immune response

Adaptive immune responses are essential in clearing or controlling HBV infection, and also crucial in mediating liver injury that drives disease progression in patients with CHB. The importance of HBV-specific immune response in the prediction of off-NA outcomes has recently been demonstrated. Rivino et al. reported that presence of HBV core and polymerase-specific T cells during NA therapy was associated with absence of flares off treatment. Comparably, increased frequency of functional HBV-specific CD8+ T cells at treatment withdrawal was associated with HBsAg loss or viral control in the study by Garcia-López et al. Rinker and colleagues also showed that the exhaustion of T cells, which is characteristic of CHB, was less severe in patients who cleared HBsAg after stopping NA.

Nevertheless, whether NA withdrawal may enhance HBV-specific T-cell immune response is unclear in view of limited and conflicting data. Moreover, the probability of functional HBV-specific CD8+ T cell response on NA treatment was found to be significantly correlated with the duration of treatment and EOT HBsAg, both of which were also significant factors associated with off-NA outcomes. Therefore, more research is needed to clarify how HBV-specific immune responses may be factored into the consideration of NA withdrawal.

Risk predictors—treatment-related factors

Treatment-related factors that may impact the risk of off NA relapses include the duration of treatment and the regimen that is discontinued. Earlier studies have shown that a short duration (<6 months) of on-therapy HBV DNA undetectability is predictive of off-therapy relapse. A large body of evidence corroborates an important role for the duration of treatment consolidation, which is defined by the period following HBeAg seroconversion in HBeAg-positive patients, or that after serum HBV DNA becomes undetectable in patients with HBeAg-negative hepatitis B, to determine the risk of off-NA relapse. In general, a minimum of one year is required for NA consolidation and extension for two or three years may be preferred. Whether further extension beyond three years confers additional benefits to protect against off-therapy relapses has not been confirmed by empirical evidence. Moreover, the types of NA therapy can impact the relapse patterns. Off-treatment relapse occurred significantly more slowly and less frequently with ETV as compared with other regimens including TDF.

Risk scores

With a few risk predictors uncovered and none sufficiently accurate by themselves, it follows that several independent risk factors may be considered together to improve the precision of risk stratification. The Japan Society of Hepatology (JSH) is the first to endorse application of a scoring formula to select candidates for treatment cessation (Table 2). The JSH score was based on serum levels of HBsAg (<80 IU/mL, 80–800 IU/mL, and >800 IU/mL scored 0, 1, and 2 points, respectively) and HBcrAg (<3 log IU/mL, 3–4 log IU/mL, and ≥4 log IU/mL scored 0, 1, and 2 points, respectively) at EOT. A total score of 3 or 4 points indicated a high risk of relapse and argued against cessation. Regrettably, external validation of the JSH score has been limited.

The SCAEL-B score is another attempt to integrate information collected at EOT to stratify the risk of clinical relapse. The scoring formula: 35*HBsAg (log IU/mL)+20*HBcrAg (log U/mL)+2*age (year)+ALT (U/L)+40 for tenofovir use (as compared with ETV) consisted of five variables that were conveniently applicable in daily practice. A total score ≥320 indicates a high risk of relapse and low chance of HBsAg seroclearance, while a score <260 points predicts the opposite. The SCALE-B score outperformed the JSH score in the development study and has been independently examined in different patient cohorts with confirmed validity in most, albeit not all, of the studies (Table 4). Nevertheless, it has not been validated in a prospective setting so far, nor has it been calibrated, and appeared insufficient to predict HBsAg seroclearance.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Race/ Region/ Setting</th>
<th>Number</th>
<th>Age (year)</th>
<th>Male (%)</th>
<th>Male/ Female</th>
<th>Pre-treatment HBeAg (+)</th>
<th>ETV/TDF proportion</th>
<th>Cirrhosis</th>
<th>Stopping rule</th>
<th>Duration (month): Treatment/ Consolidation/ Follow-up</th>
<th>Event: Clinical relapse/ HBsAg loss</th>
<th>Performance of the SCALE-B</th>
<th>Clinical relapse</th>
<th>HBsAg loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsu et al. (2019)</td>
<td>Asian/ Taiwan/ Prospective Multicenter</td>
<td>135</td>
<td>49.5*</td>
<td>80.7</td>
<td>31 (2.9%)</td>
<td>100%</td>
<td>0%</td>
<td>Treatment duration ≥3 years with undetectable HBV DNA and negative HBeAg on treatment cessation</td>
<td>36.7*/1</td>
<td>25.2*/1</td>
<td>25.9*</td>
<td>66 (48.9%)* / 8 (5.9%)</td>
<td>AUC**: 1Y: 0.87 (0.80–0.93) 3Y: 0.87 (0.79–0.94) 5Y: 0.90 (0.79–1.00) 5-year cumulative incidence**: High risk: 86.2% (67.8–96.8%) Intermediate: 61.6% (48.2–75.2%) Low risk: 17.2% (7.5–36.9%)</td>
<td>3-year incidence**: High risk: 0 Intermediate: 0 Low risk: 27.1% (14.5–47.3%)</td>
</tr>
<tr>
<td>CREATE Sonneveld et al. (2022)</td>
<td>Mixed (Asian:79.9%)/ International/ Prospective and retrospective</td>
<td>572</td>
<td>52</td>
<td>68.2</td>
<td>16.6%</td>
<td>77.8%</td>
<td>n.a.</td>
<td>Per institution</td>
<td>73.8*/1</td>
<td>n.a. / 12</td>
<td>92 (16.1%)* / 24 (4.2%)</td>
<td>Proportion at week 48§ High risk: 31% Intermediate: 14% Low risk: 3%</td>
<td>Proportion at week 48§ High risk: 1% Intermediate: 2% Low risk: 1%</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Liao et al. (2021)</td>
<td>Asian/ China/ Prospective Single center</td>
<td>122</td>
<td>34*</td>
<td>779</td>
<td>100%</td>
<td>58.2%</td>
<td>0%</td>
<td>APASL 2012</td>
<td>56.4*/1</td>
<td>30.0*/1</td>
<td>36.0*</td>
<td>44 (36.1%)* / 12 (9.8%)</td>
<td>AUC**: 1Y: 0.81 (0.73–0.89) 3Y: 0.74 (0.65–0.84) 5Y: 0.75 (0.65–0.85) 5-year cumulative incidence**: High risk: 82.2% Intermediate: 50.0% Low risk: 22.2%</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Papatheodoridis et al. (2020)</td>
<td>Caucasian/ Greece/ Prospective Multicenter</td>
<td>57</td>
<td>60</td>
<td>64.9</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>Treatment duration ≥4 years, ETV or TDF ≥2 years, and undetectable HBV DNA ≥3 years</td>
<td>n.a. / 63.6*/1</td>
<td>19.0*</td>
<td>19 (33.3%)* / 12 (21.1%)</td>
<td>No association</td>
<td>Not mentioned</td>
<td></td>
</tr>
</tbody>
</table>
PATIENT MONITORING AFTER TREATMENT CESSATION

Time patterns of off-therapy flares according to NA agents

Close monitoring after treatment is essential to detect HBV reactivation early and effectively manage acute flares, which can insidiously develop but rapidly deteriorate. How close the monitoring should be, however, is unknown. To date, no monitoring program has been prospectively validated to guarantee patient safety. There is only anecdotal and indirect evidence to support current recommendations for posttreatment monitoring.

Generally speaking, NA withdrawal may quickly reactivate HBV replication and usually result in virological relapses within three months after treatment cessation. This timing of off-therapy relapses applies to most NA agents other than ETV. For TAF, data is currently limited but it appeared similar to TDF in a pilot study. Therefore, a monthly checkup of serum HBV DNA and ALT is advisable during the first three months. If viremia substantially rebounds and/or ALT flares take place, the management should be individualized with no scheme that can fit all. In principle, subsequent monitoring should be more intense (e.g., every one to two weeks) especially when retreatment was withheld. For patients who do not discontinue ETV and do not encounter virological and/or clinical relapses in the first three months, the follow-up intensity might be decreased to every two to three months through the first year off treatment.

Although the majority of relapses take place within one year following NA withdrawal, they can still occur years afterwards. As a matter of fact, it is possible for patients to suffer HBV reactivation with resultant acute hepatitis flares as long as the viral replication remains active. Disease awareness cannot be overemphasized for NA stoppers, particularly those patients with positive indicators for a vigorous viral activity, such as substantial HBV viremia >2,000 IU/mL or high...
titers of HBsAg >1,000 IU/mL. Patients should be educated to recognize symptoms and signs suggestive of acute flares and instructed to seek medical attention as soon as possible whenever they notice or suspect the manifestations of hepatic insufficiency. Besides the due concern over acute flares and resultant complications, attention should be paid as well to more stealthy progression of the disease, such as worsening of liver fibrosis and development of HCC.

**Dynamic risk prediction in the follow-up of NA stoppers**

Posttreatment monitoring can be opportunely informed by changes in time-varying factors that foretell an upcoming bout of acute HBV flare-up. Exploration of dynamic risk predictors for posttreatment monitoring, however, has attracted far less research effort as compared to static factors at treatment initiation or EOT. Although empirical data are relatively sparse, it is conceivable that serum levels of viral DNA and HBsAg, the two convenient biomarkers that have been useful in many aspects of clinical care of HBV infection, can also be used in this application. Serum HBV DNA measured at one month after NA cessation was found to disclose early signals of imminent relapses. The investigators found that one log higher in serum HBV DNA was independent of age, biological sex, NA regimen, and EOT HBsAg level to confer a 50% (hazard ratio [HR], 1.5; 95% CI, 1.1–2.0) increase in the risk of forthcoming clinical relapse. A similar finding was reported in a recent study, which found a significant association between serum HBV DNA at week 6 off-therapy and ALT flares (ALT >5× ULN) with a HR of 1.2 (95% CI, 1.0–1.8). HBV viremia >10,000 IU/mL at week 6 was estimated to confer roughly a 3.5-fold higher risk of withdrawal flares (HR, 3.4; 95% CI, 1.4–8.4). In addition to the viral load measured at fixed time points, that of virological relapse was found to predict subsequent clinical relapse and also severe flares which required retreatment. It was shown that 89.7% (95% CI, 72.4–98.2%) of the patients with a viral load >100,000 IU/mL at the virological relapse developed clinical relapse, and 88.0% (95% CI, 68.7–97.9%) of them would need to resume treatment in two years.

How fast serum HBV DNA rises may also affect the risk of off-therapy flares. A recent retrospective study reported that a much steeper HBV DNA upsurge was associated with the risk of severe flares although the association appeared to interact with the NA regimen.

Serum level of HBsAg may markedly change following NA withdrawal in contrast to the typically unfluctuating state during continuous treatment. The time-varying HBsAg level in posttreatment follow-up can be useful to stratify the risk of subsequent relapses. Chien and colleagues confirmed that the dynamic levels of serum HBsAg was associated with forthcoming virological and clinical relapses in a prospective cohort study with serum HBsAg measured every three months after treatment cessation. They reported that the risk of subsequent clinical relapse was very low (only one in nineteen patients) with a HBsAg level below 10 IU/mL.

**CONSIDERATIONS OF THE CRITERIA TO RE-START ANTIVIRAL THERAPY**

**Dilemma over the timing of treatment resumption**

It is currently unknown and highly contentious when to resume antiviral treatment in NA stoppers who wish to give finite therapy a try. On one hand, retreatment cannot be withheld until the manifestations of jaundice or coagulopathy, let alone full-blown complications of ACLF. When acute HBV flare manifests with hepatic decompensation, it is not always rescuable by antiviral treatment and may necessitate a timely liver transplant to save lives. Even if the episode is not fatal on the spot, it may leave consequences in the long run as substantial hepatic necrosis is known to accelerate liver fibrosis toward cirrhosis with an increased risk of HCC. Therefore, immediately restarting antiviral therapy for withdrawal flares with the manifestations of liver insufficiency is certainly indicated, but is already too late to ensure patient safety.

On the other hand, recurrent viremia of itself is not an ideal indication for treatment resumption, because it is nearly universal following NA cessation. If patients want to restart treatment due to reappearance of HBV DNA in serum, they should not have interrupted the therapy in the first place. Nevertheless, it is debatable whether retreatment is indicated for recurrence of viremia with serum HBV DNA rising to a certain level with clinical implications. For instance, a viral load >2,000 IU/mL can be considered to be clinically significant and may arguably indicate antiviral therapy. Regrettably, virological relapse is still very common in patients who...
stop treatment as per the criteria of current guidelines although it is not inevitable for carefully selected candidates, such as those with a low HBsAg and/or HBcAg at EOT. If virological relapse is fiercer with a higher viral load (e.g., >100,000 IU/mL or higher), patients may consider restarting antiviral therapy, whether or not the episode is accompanied by substantial elevation of serum ALT. Withholding retreatment may not be necessary, because a fierce rebound of viremia often forecasts clinical flares that eventually require antiviral therapy. 94-96

Elevation of serum ALT as the major indication for retreatment

Rise of serum ALT may constitute the indication for retreatment following NA cessation. Various criteria have been proposed in different institutions and studies (Table 5). 34,64,102-108 Modest elevation of serum ALT (generally 1–5×ULN) in the absence of hepatic insufficiency was usually managed with close monitoring and retreatment was withheld for a certain period of time (generally one to three months). It has long been observed that serum ALT frequently fluctuated after NA withdrawal, but often could subside without retreatment. 19

Another major reason to withhold retreatment is the belief that restarting antiviral therapy may lower the chance of HBsAg seroclearance. The incidence of HBsAg seroclearance was shown to be significantly higher in patients who experienced clinical relapse and remained untreated than in those clinical relapsers who were retreated. 67 While this observation may encourage NA stoppers who are eager to clear HBsAg not to restart therapy as soon as facing mild fluctuations in serum ALT, it warrants noticing that the timing of retreatment was not randomly assigned in any study conducted to date. Whether the association is causal or confounded remains unclear, particularly when the status of exposure (i.e., clinical relapse or not, retreatment or not) is determined by events that occur after the inception of observation. Moreover, it is not clear how late is too late to avert deterioration to hepatic decompensation. Therefore, patients should not be discouraged from resuming treatment for clinical relapse, which actually meets the usual indication for treatment initiation. 9-11 For sufficiently informed patients who wish to watch and wait, intensive follow-up (every one to two weeks) is strongly advised.

In our opinion, a significant increase in serum ALT (e.g., >10 times ULN) requires immediate retreatment to reduce the risk of further progression, as acute HBV flares cannot always be predictable and the harm of delaying treatment has been well documented. 109,110 On the assumption that treating “good” flares would hinder HBsAg seroclearance, some experts have suggested withholding treatment for withdrawal flares and using the kinetics of serum HBsAg and ALT levels to guide retreatment. 111,112 However, data on the HBV-specific T cell immune response to support the practice of withholding treatment for “good” withdrawal flares is not available. In fact, existing data indicating an increase in HBV-specific adaptive immune response was not associated with withdrawal flares. 80,81 The speculation that retreatment would hinder immune reactions was based on anecdotal evidence from observational research. 16,112 Moreover, how reliably HBsAg and ALT kinetics can distinguish “good” from “bad” flares has not been prospectively attested. For the benefit of patient safety, we maintain that any proposed rule for withholding treatment in the event of acute HBV flares should be thoroughly scrutinized and validated in prospective studies before being adopted in clinical practice. After all, the consequence of an inaccurate prediction can be irreversible.

LIMITATIONS IN CURRENT KNOWLEDGE AND PERSPECTIVES FOR FUTURE RESEARCH

Interpretation of current literature to evaluate safety of NA cessation requires great caution. First of all, safety outcomes were not adequately reported and could be severely underestimated in existing studies. 42 Serious adverse events were often not specified as a study endpoint and might not have been mentioned at all. In those studies that included severe hepatitis flares or hepatic decompensation, one third (14 out of 46 reports) did not clearly define the safety events. 43 In fact, most of the previous studies focused on virological or clinical relapse and the observation could have been censored before occurrence of a more serious event. 32,42 Accordingly, we believe it is mandatory to include safety events as one of the study endpoints in every research that reports patient outcomes following NA cessation. Such events should be prespecified with a clear definition to allow for validation of the research, comparison among studies, and pooled analysis of relevant data. In addition, much of the available data originates from select specialist centers and ethnic
Table 5. Proposed criteria to restart antiviral therapy after cessation of nucleos(t)ide analogues

<table>
<thead>
<tr>
<th>Institution/Study, Site</th>
<th>Retreatment criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Criteria used in randomized controlled trials</strong></td>
<td></td>
</tr>
<tr>
<td><strong>FINITE study,</strong> Multicenter in Germany</td>
<td>At least one of the criteria:</td>
</tr>
<tr>
<td></td>
<td>· Increase of direct bilirubin by &gt;1.5 mg/dL (&gt;25 μmol/L) from baseline, and ALT &gt;ULN</td>
</tr>
<tr>
<td></td>
<td>· Increase in PT ≥2.0 s (INR ≥0.5) prolonged from baseline with adequate vitamin K therapy, and ALT &gt;ULN</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;10X ULN with or without associated symptoms.</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;2X ULN and ≤5X ULN persisting for ≥84 days (12 weeks), and HBV DNA &gt;20,000 copies/mL (equivalent to 357 IU/mL)</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;5X ULN and ≤10X ULN persisting for ≥28 days (4 weeks).</td>
</tr>
<tr>
<td><strong>Stop-NUC study,</strong> Multicenter in Germany</td>
<td>At least one of the criteria:</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;10X ULN</td>
</tr>
<tr>
<td></td>
<td>· 10X ULN ≥ALT&gt;5X ULN for ≥28 days</td>
</tr>
<tr>
<td></td>
<td>· 5X ULN ≥ALT&gt;2X ULN for ≥112 days and HBV DNA &gt;2,000 IU/mL</td>
</tr>
<tr>
<td></td>
<td>· Increase of total bilirubin by &gt;1.5X ULN</td>
</tr>
<tr>
<td><strong>Toronto-STOP study,</strong> Toronto Centre for Liver Disease, Canada</td>
<td>At least one of the criteria:</td>
</tr>
<tr>
<td></td>
<td>· HBeAg seroreversion</td>
</tr>
<tr>
<td></td>
<td>· HBV DNA &gt;2,000 IU/mL and ALT &gt;600 IU/mL at any visit</td>
</tr>
<tr>
<td></td>
<td>· HBV DNA &gt;2,000 IU/mL and ALT &gt;200 IU/mL (5X ULN) on two consecutive visits</td>
</tr>
<tr>
<td></td>
<td>· HBV DNA &gt;2,000 IU/mL and ALT &gt;200 IU/mL but &lt;600 IU/mL for &gt;6–8 weeks</td>
</tr>
<tr>
<td></td>
<td>· HBV DNA &gt;20,000 IU/mL on two consecutive visits at least 4 weeks apart.</td>
</tr>
<tr>
<td><strong>Criteria used in prospective observational study</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Queen Mary Hospital</strong>&lt;sup&gt;64&lt;/sup&gt;, Hong Kong</td>
<td>- Virological relapse: HBV DNA &gt;2,000 IU/mL</td>
</tr>
<tr>
<td><strong>Australia multicenter study,</strong>&lt;sup&gt;105&lt;/sup&gt; Australia</td>
<td>At least one of the criteria:</td>
</tr>
<tr>
<td></td>
<td>· HBV DNA &gt;2,000 IU/mL and serum ALT &gt;5X ULN for ≥16 weeks or ALT &gt;10X ULN for ≥8 weeks</td>
</tr>
<tr>
<td></td>
<td>· Clinical evidence of hepatic decompensation defined by INR ≥1.5 or bilirubin &gt;2 ULN or ascites or hepatic encephalopathy</td>
</tr>
<tr>
<td></td>
<td>· Investigator discretion</td>
</tr>
<tr>
<td><strong>DARING-B study,</strong>&lt;sup&gt;106&lt;/sup&gt; Laiko General Hospital and Hippokration General Hospital, Greece</td>
<td>At least one of the criteria:</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;10X ULN</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;5X ULN and total bilirubin &gt;2 mg/dL at the same visit</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;3X ULN and HBV DNA &gt;100,000 IU/mL at the same visit</td>
</tr>
<tr>
<td></td>
<td>· ALT &gt;ULN and HBV DNA &gt;2,000 IU/mL on three sequential visits.</td>
</tr>
<tr>
<td></td>
<td>· According to patients’ and physicians’ decisions in case of virological relapse with HBV DNA &gt;20,000 IU/mL</td>
</tr>
<tr>
<td><strong>Nanfang Hospital,</strong>&lt;sup&gt;65,107&lt;/sup&gt; China Multiple centers in China&lt;sup&gt;108&lt;/sup&gt;</td>
<td>Clinical relapse: HBV DNA &gt;2,000 IU/mL and ALT &gt;2X ULN</td>
</tr>
<tr>
<td><strong>Taiwan National Health Insurance,</strong>&lt;sup&gt;71&lt;/sup&gt; Taiwan</td>
<td>ALT &gt;2X ULN with 3 months apart and HBV DNA &gt;2,000 IU/mL or total bilirubin &gt;2 mg/dL, or prolongation of PT ≥3 seconds</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; INR, international normalized ratio; PT, prothrombin time; ULN, upper limit of normal.
groups and may not be representative of the entire population with CHB, especially those patients who receive care at primary care settings.

Moreover, existing studies were mostly retrospective and highly heterogeneous in various aspects, including compositions of patient populations, eligibility criteria for NA cessation, posttreatment monitoring, indications for retreatment, and measurements of outcomes. For instance, the proportion of patients with cirrhosis and the criteria for retreatment varied greatly among studies. Without great caution, knowledge synthesis from the literature could be inaccurate and study findings would be erroneously extrapolated to patients who were not represented in the study. In particular, there was a substantial heterogeneity in definitions. What defined a “severe” flare differed across studies. It could be a marked elevation of serum ALT alone, ALT elevation accompanied with jaundice, ALT elevation with both jaundice and coagulopathy, or overt complications of ACLF. Different definitions certainly lead to different estimates of a “nominally same” event. In order to avoid confusion and thus facilitate advancement of the knowledge, we advocate for an international consensus among leading groups or experts in the field to unify the definitions that are crucial in the studies of NA cessation.

Existing evidence so far has not confirmed the superiority of finite NA therapy over continuous treatment in improving patient outcomes. There should be tangible benefits with measurable effects to justify the risk of potential consequences following HBV reactivation in patients who elective stop the medication. Ideally, the safety, efficacy, and effect size (if effective) of a treatment strategy as compared to the standard of care needs to be evaluated by data from randomized controlled trials that are statistically powered with a representative patient population. Regrettably, evaluation of finite NA therapy is currently based predominantly on observational studies, in which the decision to stop or continue NA treatment was easily confounded. Only three randomized trials were reported to date and one of them has not been fully published. Notably, the results were inconsistent for the effect on HBsAg seroclearance (Table 6). Further evidence from robust randomized controlled trials is required for this treatment strategy to be considered standard care. After all, what was approved by regulatory agencies to treat HBV was administration of NA instead of withdrawal from it. Certainly, such a trial must be carefully planned and meticulously executed with patient safety considered as first priority.

Despite recent progress, current knowledge is insufficient for an accurate prediction of clinical outcomes in most NA stoppers. Many of the reported risk predictors have not been externally validated by data from independent study populations and thus, their generalizability is uncertain. How to accurately predict the consequences of ALT flares is particularly crucial but largely unknown. In view of the potential rapidity from ALT elevation to hepatic insufficiency, the risk of ACLF cannot be negligible unless “good” and “bad” flares can be reliably predicted before the flares. Elevation of serum ALT is already a sign of liver injury. The development of biomarkers to forecast outcomes of HBV reactivation requires deeper understanding of immune responses and resultant tissue injuries that underlie different clinical phenotypes. Regrettably, data about HBV-specific immune responses to NA withdrawal are very limited to date. Inclusion of immunological factors into the prediction models, which currently are based on viral antigens, could further improve the predictive accuracy. To this end, we believe dynamic monitoring of both viral factors (activities of replication and/or transcription) and host factors (especially adaptive immune responses) during posttreatment follow-up could be helpful and worthy of study.

**CONCLUSIONS**

Discontinuation of NA therapy leads to HBV reactivation in most of the patients who discontinued NA per the criteria recommended in guidelines. Virological and clinical relapses were common, but seroclearance of HBsAg only occurred in a minority of patients. Moreover, approximately 1% of the NA stoppers suffered severe withdrawal flares with hepatic decompensation. Accurate risk prediction is essential to identify the candidate who can safely stop NA. A useful tool is likely to include viral, host, and therapeutic factors. Close follow-up after NA cessation is mandatory, but how close the monitoring should be is not yet clear. A posttreatment surge in serum HBV DNA with a high-level viremia forecasts clinical events and may be considered an indication to restart antiviral therapy. Withholding retreatment on ALT flares cannot be recommended as a routine practice, because rapid deterioration is not always predictable and potentially devastating. In the interest of patient safety, elective discontinuation of NA thera-
<table>
<thead>
<tr>
<th>Study</th>
<th>Scale/ Region</th>
<th>Primary outcome</th>
<th>Key inclusion criteria</th>
<th>Group</th>
<th>Age/ Caucasian/ Male/ Fibroscan (kPa)</th>
<th>ETV or TDF (%)/ Pre-Tx HBsAg (+)/ HBsAg (log IU/mL)/ NA duration (months)</th>
<th>Clinical relapse, number (%)</th>
<th>HBsAg loss, number (%)</th>
<th>Adverse events, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FINITE,</strong> Berg et al.</td>
<td>Multicenter/ Germany</td>
<td>HBsAg loss or seroconversion at week 144</td>
<td>TDF ≥4 years HBV DNA &lt;400 copies/mL ≥3.5 years Pre-Tx HBeAg(-) No advanced fibrosis/cirrhosis (by histology or Fibroscan) No history of decompensation</td>
<td>Stop: n=21 44.5 / 18 (85.7%) / 6.1</td>
<td>21 (100%)/ 0 (0%)/ n.a.</td>
<td>At least 5 (23.8%)††</td>
<td>4 (19.0%)</td>
<td>Grade 3/4: 5 (23.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Continue: n=21 45.5 / 19 (90.5%)/ 15</td>
<td>21 (100%)/ 0 (0%)/ n.a.</td>
<td>1 (4.8%)**††</td>
<td>0 (0%)</td>
<td>Grade 3/4: 0 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Stop-NUC,</strong> van Bömmel et al.</td>
<td>Multicenter/ Germany</td>
<td>HBsAg loss at week 96</td>
<td>NA ≥4 years HBV DNA &lt;1,000 IU/mL ≥4 years Pre-Tx HBeAg(-) Pre-Tx HBV DNA &gt;2,000 IU/mL No advanced fibrosis/cirrhosis (by histology of Fibroscan)</td>
<td>Stop: n=79 51.6 / 62 (78.5%)/ 5.7</td>
<td>71 (89.9%)/ 3.5 / n.a.</td>
<td>28 (35.4%)†</td>
<td>10 (12.7%)</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Continue: n=79 52.0 / 68 (82.2%)/ 5.1</td>
<td>72 (91.1%)/ 0 / n.a.</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td><strong>Toronto-STOP,</strong> Liem et al.</td>
<td>Single center/ Canada</td>
<td>HBV DNA &lt;2,000 IU/mL at week 48</td>
<td>NA ≥1 year Consolidation: Pre-Tx HBeAg(+): 1 year and HBeAb(-) Pre-Tx HBV DNA &gt;2,000 IU/mL No cirrhosis (defined by histology or Fibroscan)</td>
<td>Stop: n=45 59 / 26 (57.8%)/ 4.9</td>
<td>45 (100%)/ 3.1 / 7.2</td>
<td>At least 10 (22.2%)†</td>
<td>1 (0.2%)/ 2 (4.9%): ALT &gt;5X ULN</td>
<td>1 (2.2%): bilirubin &gt;66 μmol/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Consolidation: n=22 50 / 14 (63.6%)/ 5.2</td>
<td>22 (100%)/ 13 (59.1%)/ 3.0</td>
<td>0 (0%)/ 1 (0.5%)/ 0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; ETV, entecavir; HBeAb, anti hepatitis B e-antibody; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; n.a., not available; ULN, upper limit of normal; TDF, tenofovir disoproxil fumarate; Tx, treatment.

†Abstract, not full-length article, †mean, †defined by ALT >3X ULN, †defiend by ALT >1.5X ULN and HBV DNA >2,000 IU/mL, ††data at week 72,**defined by ALT > 2X ULN,**according to the figure of the paper.
A safe and practical protocol for off-NA follow-up not yet clear
For NAs other than ETV, close monitoring (e.g., monthly) in the first 3 months and then maybe less frequent (e.g., every 2-3 months)
For ETV, closer monitoring may commence after 3 months off NA
Follow-up intensity may decrease to every 3-6 months after two-year monitoring without occurrence of relapses
Serum HBV DNA essential in posttreatment monitoring

A high HBV DNA level and/or ALT elevation indicate the need of intensive monitoring (e.g., every 1-2 weeks) or retreatment
Patients should be made alert to symptoms and signs of hepatic decompensation

**Patient selection**

- Highly motivated individuals fully informed of potential risks
- Guaranteed adherence to posttreatment monitoring
- Consolidation treatment at least ≥1 year (preferably 3 years)
- No cirrhosis/advanced fibrosis or history of liver failure
- A favorable benefit-risk profile according to validated predictors (e.g., EOT HBsAg <100 IU/mL, SCALE-B score <260 points)

**Posttreatment monitoring**

- Immediate retreatment for severe ALT flares or manifestations of hepatic insufficiency
- May restart treatment for a steep rise of viremia and/or modest ALT elevation (2-5 x ULN)
- Withholding treatment on ALT flares unadvisable outside a research setting with informed consent

**Occurrence of virological or clinical relapses**

- A high HBV DNA level and/or ALT elevation indicate the need of intensive monitoring (e.g., every 1-2 weeks) or retreatment
- Patients should be made alert to symptoms and signs of hepatic decompensation

**Retreatment**

- A safe and practical protocol for off-NA follow-up not yet clear
- For NAs other than ETV, close monitoring (e.g., monthly) in the first 3 months and then maybe less frequent (e.g., every 2-3 months)
- For ETV, closer monitoring may commence after 3 months off NA
- Follow-up intensity may decrease to every 3-6 months after two-year monitoring without occurrence of relapses
- Serum HBV DNA essential in posttreatment monitoring

**Figure 2.** Safety considerations along the proposed scheme for an elective cessation of nucleos(t)ide analogues. ALT, alanine aminotransferase; DNA, DNA, deoxyribonucleic acid; EOT, end of treatment; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, nucelos(t)ide analogue; RNA, ribonucleic acid; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.


20. Lampertico P, Berg T. Less can be more: A finite treatment approach for HBeAg-negative chronic hepatitis B. Hepatology 2018;68:397-400.


35. Liu YC, Jeng WJ, Peng CW, Chien RN, Liaw YF. Off-tenofovir hepatitis flares in HBeAg-negative patients occur earlier, more frequent and severe than those off-entecavir therapies. Liver Int 2022;42:551-560.


50. Höner Zu Siederdissen C, Rinker F, Maasoumy B, Wiegand SB, Filmann N, et al. Viral and host responses after stopping...
long-term nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. J Infect Dis 2016;214:1492-1497.


73. Chi H, Li Z, Hansen BE, Yu T, Zhang X, Sun J, et al. Serum level of


95. Hsu YC, Mo LR, Chang CY, Wu MS, Yang TH, Kao JH, et al. Serum viral load at the virological relapse predicts subsequent clini-


105. Hall SAL, Burns GS, Anagnostou D, Vogrin S, Sundararajan V, Ratnam D, et al. Stopping nucleos(t)ide analogues in non-cirrhotic HBeAg-negative chronic hepatitis B patients: HBsAg loss at 96 weeks is associated with low baseline HBsAg levels. Aliment Pharmacol Ther 2022;56:310-320.


108. Xie Y, Li M, Ou X, Zheng S, Gao Y, Xu X, et al. HBeAg-positive patients with HBsAg <100 IU/mL and negative HBV RNA have lower risk of virological relapse after nucleos(t)ide analogues cessation. J Gastroenterol 2021;56:856-867.


111. Liaw YF, Jeng WJ, Chang ML. HBsAg kinetics in retreatment decision for off-therapy hepatitis B Flare in HBeAg-negative patients. Gastroenterology 2018;154:2280-2281.


Hepatorenal syndrome: Current concepts and future perspectives

Chan-Young Jung and Jai Won Chang
Division of Nephrology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Hepatorenal syndrome (HRS), a progressive but potentially reversible deterioration of kidney function, remains a major complication in patients with advanced cirrhosis, often leading to death before liver transplantation (LT). Recent updates in the pathophysiology, definition, and classification of HRS have led to a complete revision of the nomenclature and diagnostic criteria for HRS type 1, which was renamed HRS-acute kidney injury (AKI). HRS is characterized by severe impairment of kidney function due to increased splanchnic blood flow, activation of several vasoconstriction factors, severe vasoconstriction of the renal arteries in the absence of kidney histologic abnormalities, nitric oxide dysfunction, and systemic inflammation. Diagnosis of HRS remains a challenge because of the lack of specific diagnostic biomarkers that accurately distinguishes structural from functional AKI, and mainly involves the differential diagnosis from other forms of AKI, particularly acute tubular necrosis. The optimal treatment of HRS is LT. While awaiting LT, treatment options include vasoconstrictor drugs to counteract splanchnic arterial vasodilation and plasma volume expansion by intravenous albumin infusion. In patients with HRS unresponsive to pharmacological treatment and with conventional indications for kidney replacement therapy (KRT), such as volume overload, uremia, or electrolyte imbalances, KRT may be applied as a bridging therapy to transplantation. Other interventions, such as transjugular intrahepatic portosystemic shunt, and artificial liver support systems have a very limited role in improving outcomes in HRS. Although recently developed novel therapies have potential to improve outcomes of patients with HRS, further studies are warranted to validate the efficacy of these novel agents. (Clin Mol Hepatol 2023;29:891-908)

Keywords: Hepatorenal syndrome; Liver cirrhosis; Acute kidney injury; Biomarkers; Terlipressin

INTRODUCTION

Hepatorenal syndrome (HRS) is a serious complication of end-stage cirrhosis and portal hypertension that is characterized by increased splanchnic blood flow, a state of decreased central volume, kidney blood flow and glomerular filtration rate (GFR).¹ Hepatorenal syndrome is typically diagnosed when there is a marked reduction in GFR, and in the absence of evidence of intrinsic kidney diseases, such as hematuria, proteinuria, or abnormal kidney ultrasonography. This is contrary to what occurs in most cases of intrinsic kidney damage, in which there are marked changes in kidney histology.¹² This review focuses on several conceptual issues that have emerged in the hepatorenal field.

Corresponding author: Jai Won Chang
Division of Nephrology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-3260, Fax: +82-2-3010-6963, E-mail: jwchang@amc.seoul.kr
https://orcid.org/0000-0003-0296-5992
DEFINITION OF HEPATORENAL SYNDROME

The definition of HRS has significantly evolved over the past several decades (Table 1). In 1996, the International Club of Ascites (ICA) defined acute kidney injury (AKI) in cirrhosis as an increase in serum creatinine of ≥50% from baseline to ≥1.5 mg/dL. Other important components of AKI in cirrhosis included oliguria, as well as proteinuria <500 mg/dL. In 2007, HRS was further classified into two types: type 1, characterized by a rapid deterioration of kidney function by doubling of initial serum creatinine to ≥2.5 mg/dL or a 50% reduction in less than 2 weeks in the initial 24-hour creatinine clearance to below 20 mL/min that often occurs due to a precipitating event; and type 2, in which kidney failure progression did not meet the criteria for type 1. Importantly, urinary sodium and oliguria were removed from the new diagnostic criteria. Several studies indicating that the diagnosis of AKI in patients with cirrhosis, based on an absolute increase in serum creatinine by ≥0.3 mg/dL or 50% from baseline, leads to earlier identification of patients with poorer outcomes led to the ICA to revise the definition of HRS in 2015, incorporating a new definition and classification of AKI with modifications (Table 2). Serum creatinine obtained in the previous 3 months can be used as baseline when a baseline level obtained during the previous 7 days is not available. Although oliguria was not included in the definition of AKI in patients with cirrhosis, a study indicating that urine output was found to be significantly associated with adverse outcomes in patients with AKI and cirrhosis led to calls for a new definition and overall a new classification for HRS that expands on the 2015 ICA consensus document. Most recently, the ICA completely revised the nomenclature and diagnostic criteria for HRS type 1, which is now called HRS-AKI. Results of several studies showed that the higher the initial serum creatinine level at the start of treatment, the lower the probability of HRS reversal. This led to the ICA removing the minimum creatinine value for diagnosis, and therefore HRS-AKI can be diagnosed even when the serum creatinine level is below 2.5 mg/dL. Functional kidney injury that does not meet the criteria of HRS-AKI is termed HRS-NAKI (i.e., non-AKI), of which NAKI is further divided into HRS-acute kidney disease (HRS-AKD) if the estimated glomerular filtration rate (eGFR) is below 60 mL/min/1.73m² for less than 3 months and HRS-chronic kidney disease (HRS-CKD) if eGFR is below 60 mL/min/1.73m² for more than 3 months.

PATHOPHYSIOLOGY

The pathophysiology of HRS is characterized by reduced systemic vascular resistance due to splanchnic arterial vasodilation, which occurs secondary to portal hypertension, a key feature of advanced cirrhosis. However, recent studies have suggested that a systemic inflammatory state may lead to an increase in the release of inflammatory mediators, and therefore may play a role in the circulatory and kidney dysfunction observed in HRS. Therefore, it is now recognized that HRS not only involves circulatory dysfunction but also systemic inflammation. (Fig. 1).

Circulatory dysfunction

End-stage liver disease resulting in cirrhosis leads to increased intrahepatic vascular resistance, which subsequently causes splanchnic vasodilation triggered by increased production of vasodilators including nitric oxide, prostacyclins, carbon monoxide, and endocannabinoids. Splanchnic vasodilation subsequently leads to decreased vascular resistance and reduced effective arterial blood volume (EAVB). Although the heart is able to compensate for this decrease in EAVB in the early stages of cirrhosis by increasing cardiac output, but subsequent development of cirrhotic cardiomyopathy, aggravation of portal hypertension and splanchnic vasodilation results in effective arterial hypovolemia and arterial hypotension. This decrease in EAVB subsequently activates various vasoconstriction factors that include the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (SNS), tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and endogenous vasoconstrictors such as endothelin, angiotensin II, and vasopressin. The resulting decrease in cardiac output leads to a decreased renal blood flow (RBF), which in turn leads to decreased glomerular filtration rate (GFR) and increased urine sodium and potassium excretion. This feedback loop further exacerbates the decrease in cardiac output, leading to a downward spiral of decreasing cardiac output and progressive decrease in GFR.

Abbreviations:

ADMA, asymmetric dimethylarginine; AKD, acute kidney disease; AKI, acute kidney injury; ATN, acute tubular necrosis; CKD, chronic kidney disease; CKRT, continuous kidney replacement therapy; DAMP, damage-associated molecular pattern; EAVB, effective arterial blood volume; eGFR, estimated glomerular filtration; FeNa, fractional excretion of sodium; GFR, glomerular filtration rate; HRS, hepatorenal syndrome; ICA, International Club of Ascites; IL-6, interleukin-6; IV, intravenous; KRT, kidney replacement therapy; LT, liver transplantation; MARS, molecular adsorbent recirculating system; NAKI, non-AKI; NGAL, neutrophil gelatinase associated lipocalin; NO, nitric oxide; NOS, nitric oxide synthase; PAMP, pathogen-associated molecular pattern; RAA, renin-angiotensin-aldosterone system; RBF, renal blood flow; SBP, spontaneous bacterial peritonitis; SDMA, symmetric dimethylarginine; SKLT, simultaneous liver-kidney transplantation; SNS, sympathetic nervous system; TIPS, transjugular intrahepatic portosystemic shunt; TLR, toll-like receptor; TNF-α, tumor necrosis factor α
Table 1. Previous and current definitions of hepatorenal syndrome

<table>
<thead>
<tr>
<th>Criteria</th>
<th>1996\textsuperscript{3}</th>
<th>2007\textsuperscript{4}</th>
<th>2015\textsuperscript{12}</th>
<th>2019\textsuperscript{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine and/or change in serum creatinine</td>
<td>Serum creatinine ≥1.5 g/dL OR 24-hr Creatinine clearance &lt;40 mL/min</td>
<td>Serum creatinine ≥1.5 mg/dL OR Doubling of serum creatinine to ≥2.5 mg/dL within 2 weeks</td>
<td>Increase in serum creatinine by ≥0.3 mg/dL within 48 hours OR Increase in serum creatinine ≥1.5 times from baseline (Creatinine value within previous 3 months, when available, may be used as baseline, and value closest to presentation should be used) within 7 days</td>
<td>Rename HRS-1 to HRS-AKI Increase in serum creatinine by ≥0.3 mg/dL within 48 hours OR Increase in serum creatinine ≥1.5 times from baseline (Creatinine value within previous 3 months, when available, may be used as baseline, and value closest to presentation should be used) within 7 days</td>
</tr>
<tr>
<td>Urinary sodium</td>
<td>&lt;10 mEq/L</td>
<td>-</td>
<td>-</td>
<td>FENa &lt;0.2%</td>
</tr>
<tr>
<td>Urine volume</td>
<td>&lt;500 mL/day</td>
<td>-</td>
<td>&lt;0.5 mL/kg/hr for 6 hours</td>
<td>&lt;0.5 mL/kg/hr for 6 hours</td>
</tr>
<tr>
<td>Urine sediment</td>
<td>Absence of structural kidney damage, as defined by proteinuria &lt;500 mg/dL and/or urine RBC &lt;50/HPF</td>
<td>Absence of structural kidney damage, as defined by proteinuria &lt;500 mg/dL and/or urine RBC &lt;50/HPF</td>
<td>Absence of structural kidney damage, as defined by proteinuria &lt;500 mg/dL and/or urine RBC &lt;50/HPF</td>
<td>Absence of structural kidney damage, as defined by proteinuria &lt;500 mg/dL and/or urine RBC &lt;50/HPF and/or NGAL &lt;220 μg/g Cr</td>
</tr>
<tr>
<td>Miscellaneous criteria</td>
<td>1. Cirrhosis or acute liver disease with portal hypertension 2. Absence of shock, ongoing bacterial infection, or current/recent treatment with nephrotoxic drugs</td>
<td>1. Cirrhosis or acute liver disease with portal hypertension 2. Absence of shock, ongoing bacterial infection, or current/recent treatment with nephrotoxic drugs</td>
<td>1. Cirrhosis or acute liver disease with portal hypertension 2. Absence of shock, ongoing bacterial infection, or current/recent treatment with nephrotoxic drugs</td>
<td>1. Cirrhosis or acute liver disease with portal hypertension 2. Absence of shock, ongoing bacterial infection, or current/recent treatment with nephrotoxic drugs</td>
</tr>
</tbody>
</table>

RBC, red blood cell; HRS-1, hepatorenal syndrome type 1; HRS-AKI, hepatorenal syndrome-acute kidney injury; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase associated lipocalin.
The angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS), and the non-osmotic secretion of arginine vasopressin are vasoconstrictor factors that assist in maintaining arterial pressure near normal limits. Although these vasoconstrictor factors activate hemodynamic effects on kidney function, resulting in renal vasoconstriction, impaired solute-free water excretion and subsequent decline in kidney function. The kidneys are also able to compensate for such changes during earlier stages, owing to the vasodilatory effects of renal prostaglandins (prostaglandins E2 and I2), which maintain glomerular pressure despite reduced renal blood flow (RBF). Progression of liver disease and the use of concomitant non-steroidal anti-inflammatory drugs that inhibit prostaglandin synthesis disrupts this balance and, therefore, causes AKI.

Diastolic dysfunction may be present in up to 60% of patients with cirrhosis; however, the relationship between diastolic dysfunction and development of HRS has not been demonstrated. Marked renal vasoconstriction in patients with HRS has been demonstrated in a number of studies. The pathophysiological hallmark of HRS is its vasogenic nature. Marked renal vasoconstriction in patients with HRS has been demonstrated in a number of studies. The pathophysiological hallmark of HRS is its vasogenic nature. Two major vasoconstrictor systems that act on renal circulation are the sympathetic nervous system (SNS) and the non-osmotic secretion of arginine vasopressin. Although these vasoconstrictor factors activate hemodynamic effects on kidney function, resulting in renal vasoconstriction, impaired solute-free water excretion and subsequent decline in kidney function. The kidneys are also able to compensate for such changes during earlier stages, owing to the vasodilatory effects of renal prostaglandins (prostaglandins E2 and I2), which maintain glomerular pressure despite reduced renal blood flow (RBF). Progression of liver disease and the use of concomitant non-steroidal anti-inflammatory drugs that inhibit prostaglandin synthesis disrupts this balance and, therefore, causes AKI.

Table 2. Stages of acute kidney injury according to the International Club of Ascites

<table>
<thead>
<tr>
<th>AKI stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Increase in serum creatinine ≥0.3 mg/dL or increase in serum creatinine ≥1.5-fold to twofold from baseline*</td>
</tr>
<tr>
<td>Stage 1A</td>
<td>Serum creatinine &lt;1.5 mg/dL†</td>
</tr>
<tr>
<td>Stage 1B</td>
<td>Serum creatinine ≥1.5 mg/dL</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Increase in serum creatinine at least twofold to threefold from baseline</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Increase in serum creatinine at least threefold from baseline or serum creatinine ≥4.0 mg/dL with an acute increase ≥0.3 mg/dL or initiation of kidney replacement therapy</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury.

*Baseline serum creatinine is defined as a value of serum creatinine obtained in the previous 3 months. In patients with more than one value obtained within the previous 3 months, the value closest to admission time to hospital should be used. In patients without a previous serum creatinine value, the serum creatinine on admission should be used as baseline.

†AKI stages 1A and 1B are adaptations of the International Club of Ascites definitions of AKI stages by the European Association for the Study of the Liver.
in this pathophysiological process is the RAAS and the SNS. In several studies of patients with cirrhosis, activity of the RAAS, as estimated by plasma renin activity, was shown to increase from compensated to decompensated cirrhosis. Peak activity was seen in patients with HRS and it was shown to correlate inversely with kidney function. Moreover, in patients with infection associated HRS, patients with higher RAAS activity had a significantly lower probability of HRS reversal than those with lower RAAS activity. Plasma levels of norepinephrine, which reflects SNS activity, are increased in patients with HRS than in those with ascites and intact kidney function, and were shown to be inversely correlated with GFR. However, considering that both RAAS and SNS are two vasoconstrictor systems that act to increase arterial blood pressure and counteract splanchnic vasodilation, studies have been unable to assess whether the blockade of these RAAS and SNS lead to improved outcomes in patients with cirrhosis. Other than the aforementioned vasoconstrictor systems, other factors with a potential role in kidney vasoconstriction in HRS include endothelin, cysteinyl leukotrienes, and prostaglandins.

**Nitric oxide dysfunction**

In cirrhosis, a reduction in RBF is also partly due to either excessive or insufficient nitric oxide (NO) production. Excess NO production results in splanchnic vasodilation, reduced EABV, RAAS, and SNS activation, and renal vasoconstriction. However, insufficient NO release may also cause reduced RBF. This may be partly due to the increased production of dimethylarginines, such as symmetric (SDMA) and asymmetric dimethylarginine (ADMA). ADMA levels are increased in advanced liver disease, and therefore NO synthesis from NO synthase (NOS) is inhibited, and therefore RBF is compromised. SDMA, an ADMA isomer, also increases in the setting of decreased hepatic and kidney function. High concentrations of SDMA also reduces NO production, resulting in reduced RBF. This has led to some studies indicating that SDMA may be a potential marker for HRS.Indeed, several studies have indicated that both SDMA and ADMA are...
potential independent predictors of measured GFR in cirrhotic patients.\textsuperscript{41}

Systemic inflammation

It is now recognized that systemic inflammation also plays a part in HRS pathophysiology. Systemic inflammatory response syndrome has been observed in almost half of patients with HRS-AKI, independent of the presence of actual infection.\textsuperscript{45} In particular, those with the most extensive baseline systemic inflammation also had the highest risk of liver failure development and mortality.\textsuperscript{46} Plasma levels of pro-inflammatory cytokines (interleukin-6 [IL-6] and tumor necrosis factor-\textalpha{} [TNF-\textalpha{]}), and urinary levels of monocyte chemoattractant protein-1 are increased in patients with HRS-AKI than in those with decompensated cirrhosis without AKI and those with AKI secondary to pre-renal azotemia.\textsuperscript{47}

The main mechanism by which the systemic inflammatory state primarily contributes to the pathogenesis of HRS is the translocation of gut bacteria from the gut to mesenteric lymph nodes due to altered intestinal permeability.\textsuperscript{48} This bacterial translocation not only induces increased levels of pro-inflammatory cytokines,\textsuperscript{49} in particular IL-6 and TNF-\textalpha{},\textsuperscript{50} but also increased levels of various vasodilating factors, such as NO,\textsuperscript{51} which contribute to the decreased EABV, as well as a wide spectrum of molecules (pathogen-associated molecular patterns [PAMPs] and damage-associated molecular patterns [DAMPs]) that are responsible for inducing inflammatory responses through pattern recognition receptors such as toll-like receptors (TLRs). PAMPs are products of bacteria that include lipopolysaccharide, flagellin, and nigericin, whereas DAMPs are intracellular components released from injured hepatocytes that include high-mobility group protein B1, heat shock proteins, and hyaluronic acid. Not only are both PAMPs and DAMPs known to have systemic effects by promoting inflammation and the release of pro-inflammatory cytokines, but both molecules may also have direct effects on the kidney. For example, in a study of patients with kidney dysfunction and cirrhosis, patients showed increased renal expression and urinary excretion of TLR4, suggesting a potential role of TLR4 as a mediator of kidney injury.\textsuperscript{52} Moreover, gut decontamination in rodent models of cirrhosis has been shown to reduce renal TLR4 expression and subsequently prevent kidney dysfunction, suggesting that exposure to PAMPs from gut bacterial translocation may increase TLR4 expression in the kidneys.\textsuperscript{53}

Differential diagnosis and biomarkers

As the treatment of AKI in patients with cirrhosis depends on the type of AKI, determining the etiology is essential.\textsuperscript{25,54} Although the differential diagnosis of AKI in patients with cirrhosis is broadly similar to that in other patient populations, the differential diagnosis is often not so straightforward. In addition to HRS, other types of AKI that can occur include volume-responsive pre-renal AKI due to infection, hypovolemia, vasodilators, obstructive post-renal AKI, and intra-renal AKI that may be caused by toxin or ischemia induced acute tubular necrosis (ATN), or glomerulonephritis. Considering that patients with ATN and HRS have the worst survival among those with AKI and cirrhosis,\textsuperscript{55} accurate differential diagnosis of the etiology is important.

HRS remains a diagnosis of exclusion. A key component in HRS diagnosis is exclusion of structural kidney damage, which relies on urine microscopy and urine sodium excretion. Other requirements include the absence of shock, proteinuria (>500 mg/day), and microscopic hematuria (>50 red blood cells per high power field), along with normal kidney morphology on ultrasonography. However, possibly due to systemic inflammation that can also cause ATN, differentiating between ATN and HRS is often very difficult. Although urinary sodium (>40 mEq/L), fractional excretion of sodium (FeNa >2%), and low urine osmolality (<400 mOsm/L) are suggestive of ATN, other conditions, such as the use of diuretics that are commonly used in patients with large volume ascites, may confound the interpretation of FeNa.\textsuperscript{56} Moreover, low FeNa was also found in biopsy proven-ATN,\textsuperscript{57} and therefore urinary sodium and FeNa are no longer part of the most recent diagnostic criteria of HRS-AKI.\textsuperscript{14} A more useful marker in differentiating between ATN and HRS may be the fractional excretion of urea,\textsuperscript{58,59} because unlike sodium, reabsorption of urea occurs primarily in the proximal tubules of the kidney, and therefore is not affected by commonly used diuretics such as loop diuretics and spironolactone, which act in the loop of Henle and distal convoluted tubules, respectively.

Several novel biomarkers that may be useful in the differential diagnosis of AKI in patients with cirrhosis have recently been investigated.\textsuperscript{57,60} Most of these biomarkers originate from kidney tubular proteins released during cell damage,
upregulated during kidney injury, proteins with diminished tubular reabsorption, and markers of inflammation. Of the above markers, to date, the most widely investigated is urinary neutrophil gelatinase associated lipocalin (NGAL). In a multicenter, prospective cohort study involving 188 patients with AKI and cirrhosis, median values of urinary NGAL, IL-18, kidney injury molecule-1, liver-type fatty acid binding protein, and albumin were all elevated in patients with ATN. In another study involving 241 patients with cirrhosis, urinary NGAL levels were markedly higher in patients with ATN than in those with pre-renal azotemia, CKD, and HRS. In a more recent study involving 320 patients with AKI hospitalized for decompensated cirrhosis, urinary NGAL measured at day 3 had the greatest accuracy for differential diagnosis between ATN and other etiologies of AKI.

Not only are biomarkers important for the differential diagnosis of AKI, but they may also play an important role in predicting treatment response of HRS, and even for prognosis. For example, in 162 patients with AKI and cirrhosis, not only was urinary NGAL an adequate biomarker in the differential diagnosis of AKI, but it also predicted the response to terlipressin and albumin in patients with HRS-AKI, and was also an independent predictor of in-hospital mortality. Similarly, in a study consisting of 213 United States (US) hospitalized patients with decompensated cirrhosis, not only did urinary NGAL differentiate the type of AKI in cirrhosis, but also significantly predicted 90-day transplant-free survival, and outperformed Model for End-Stage Liver Disease score in terms of survival prediction.

Although the most ideal biomarker would be one that distinguishes structural from functional AKI, but in reality, no biomarkers to date perform optimally in the differential diagnosis of AKI in patients with cirrhosis. Further validation studies are warranted for their generalized applications.

RISK FACTORS AND PREVENTION

The most common risk factors for HRS are those related to systemic inflammation and acute hemodynamic changes. Therefore, the most commonly known precipitants of HRS are SBP, other systemic infections, and large volume paracentesis without albumin administration. HRS develops in as many as 30% of patients with SBP, and is associated with significantly worse outcomes. Infection-associated HRS may be prevented by administration of intravenous (IV) albumin in addition to antibiotic treatment in the setting of SBP and may also reduce overall mortality. In the setting of SBP, IV albumin may be administered 1.5 g/kg on day 1 followed by 1 g/kg on day 3. In patients undergoing large-volume paracentesis (>5 L), albumin administration has been shown to decrease the incidence of HRS. However, data on whether albumin prevents HRS or improves overall survival has been conflicting. For example, in the ANSWER (human Albumin for the treatment of ascites in patients with hepatic cirrhosis) study, long-term administration of human albumin was associated with improved overall 18-month survival compared to standard medical treatment. However, in the MACHT (midodrine and albumin for cirrhotic patients in the waiting list for liver transplantation) study, treatment with midodrine and albumin failed to prevent complications of cirrhosis or improve survival. Most recently, in the ATTIRE (Albumin to Prevent Infection in Chronic Liver Failure) trial that investigated whether higher doses of albumin therapy to increase and maintain serum albumin levels to 30 g/L or more improved outcomes in hospitalized patients with cirrhosis, the results were largely disappointing, and therefore, supporting the need for a re-evaluation of the use of albumin in patients with cirrhosis. The ongoing PRECiosa12 (Effects of Long Term Administration of Human Albumin in Subjects With Decompensated Cirrhosis and Ascites) trial will hopefully clarify the role of long term albumin use in this population.

β-blockers are effective in preventing variceal bleeding, which can precipitate HRS. Although they are widely used in patients with cirrhosis and portal hypertension, therapy must be individualized based on the severity of hepatic decompensation. In patients with compensated cirrhosis, treatment with β-blockers was associated with a preservation in kidney function, and an increase in decompensation-free survival, mainly by reducing the incidence of ascites. However, in patients with decompensated cirrhosis with ascites, reports have been conflicting. While a recent meta-analysis suggested that the use of β-blockers in patients with cirrhosis and ascites was not associated with a significant increase in mortality, some reports have suggested that the decrease in cardiac output caused by β-blockers could precipitate AKI, and therefore increase mortality in this patient group. Therefore, clinicians should carefully weigh the risks and benefits of continuation of β-blockers in patients.
with cirrhosis.

In patients with ascites and a high risk of developing SBP, as determined by a low ascitic fluid protein (<1.5 g/dL), concomitant advanced liver failure (Child-Pugh score ≥9 points with serum bilirubin level ≥3 mg/dL) or kidney dysfunction (serum creatinine level ≥1.2 mg/dL, blood urea nitrogen level ≥25 mg/dL, or serum sodium level ≤130 mEq/L), antibiotic prophylaxis using either norfloxacin or rifaximin has shown to prevent the development of SBP and HRS, as well as reduce overall mortality.88-91

**TREATMENT OF HEPATORENAL SYNDROME**

The management of AKI in patients with cirrhosis should begin immediately once a diagnosis has been made and the etiology of AKI identified because patients with AKI and cirrhosis often deteriorate rapidly (Fig. 2). Once a diagnosis of AKI has been made, management of AKI should typically begin with a fluid challenge of 20–25% IV albumin at 1 g/kg/day for 2 days and withdrawal of any diuretics (expert opinion, not evidence-based).12 Low volume therapeutic paracentesis with albumin to control ascites should also be performed if necessary.82,83 This not only rules out pre-renal azotemia, but also promotes early circulating volume expansion in the context of reduced EABV. The initial phase of treatment also consists of temporary discontinuation of non-selective β-blockers given their negative inotropic effect,84,85 and other potential nephrotoxic agents and vasodilators.

**Pharmacologic therapy**

**Vasoconstrictors**

The rationale behind the use of vasoconstrictors in the treatment of HRS is to counteract splanchnic arterial vasodilation.12,86 Of the currently available vasoconstrictors, terlipressin, a synthetic vasopressin analog with a predominant vasopressin 1A receptor effect acting primarily as a splanchnic vasoconstrictor,87 is the most commonly used vasopressin analog.

To date, terlipressin is the vasopressin with the most convincing data to date.88 Several positive results from clinical trials have led to the US Food and Drug Administration recently approving the use of terlipressin in the US for improving kidney function in patients with HRS. Terlipressin is generally administered by IV boluses at starting doses of 0.5–1 mg every 4–6 hours. The dose can be increased to a maximum of 2 mg every 4 hours in cases of nonresponse, defined as less than a 25% reduction in serum creatinine level after 3 days and no side effects occur.89-91 Doses should be maintained for a maximum of 14 days depending on responses to treatment. Doses of terlipressin in combination with albumin should be continued until serum creatinine reaches a final value <1.5 mg/dL, or until baseline creatinine level. If patients show nonresponse or partial response, the treatment should be discontinued within 14 days. The efficacy of continuous infusion of terlipressin has been supported in a single center study of 78 patients, where continuous IV terlipressin infusion was shown to be not only better tolerated than IV boluses, but was also effective at doses lower than those required for IV bolus administration.92 The INFUSE (Terlipressin for HRS-AKI in Liver Transplant Candidates) trial that will evaluate the use of continuous terlipressin infusion in patients on the liver transplant waiting list with HRS-AKI is currently ongoing.93

According to two previous randomized controlled clinical trials performed in Europe, the response rate of terlipressin plus albumin is around 50%.92,94 Most recently, the efficacy and safety of terlipressin plus albumin for the treatment of type 1 HRS have been proven in a multicenter phase 3 trial, in which enrolled patients were randomly assigned in a 2:1 ratio to receive terlipressin or placebo for up to 14 days. In this trial involving 300 patients with cirrhosis and type 1 HRS, terlipressin was more effective than placebo in improving kidney function.95 Despite the high response rates of terlipressin plus albumin, recurrence of HRS is not uncommon, with recurrences occurring in <20% of patients with type 1 HRS. These patients may be re-treated with vasoconstrictors and albumin. The patient’s response to terlipressin is not only important for HRS reversal, but it has also shown to be an important prognostic factor in liver transplantation (LT) patients.96 For example, in two cohorts of patients with cirrhosis listed for LT, one with and one without HRS-AKI, response to terlipressin and albumin reduced the need for KRT after LT, and also reduced the risk of CKD at 1 year after LT.98 Factors associated with lower response to terlipressin and albumin include higher baseline serum creatinine, urinary NGAL and serum bilirubin, lower increases in arterial pressure, presence of systemic inflammatory response syndrome, and more severe acute on chronic liver failure grade.95,97-99
Common side effects of terlipressin include diarrhea and abdominal pain, which are reported in around 10–20% of patients. More serious side effects are related to vasoconstriction with a risk of myocardial infarction and intestinal ischemia, with a rate of 2–13%. In the recent CONFIRM trial, the use of terlipressin was also associated with a higher risk of respiratory failure. Patients on terlipressin should be monitored for signs of ischemia while on therapy, and the drug should be avoided in patients with a history of coronary artery disease or peripheral artery disease. Furthermore, as the response to treatment is attenuated in patients with higher degrees of kidney injury and acute on chronic liver failure grade, the risk-benefit of administering vasoconstrictors in combination with IV albumin should be carefully considered.

Other vasoconstrictor treatment options include norepinephrine, and the combination of midodrine and octreotide. Norepinephrine is a systemic vasoconstrictor that acts through the activation of α-1 adrenergic receptors on vascular smooth muscle cells. Norepinephrine is administered at 0.5–3 mg/h continuous IV infusion, titrating dosing to achieve an increase of 10 mmHg in mean arterial pressure. Norepinephrine in combination with albumin is also effective and safe, with response rates ranging from 39–70%. It is a cheaper drug than terlipressin; however, unlike terlipressin, which can be administered peripherally, norepinephrine can only be administered through a central venous line. When using norepinephrine, close monitoring for tachyarrhythmias or bradycardia is needed.

A combination of midodrine, an α-adrenergic agonist, plus octreotide, a somatostatin analogue, may also be used. Mid-
odrine is administered as 7.5 mg up to 12.5 mg orally three times a day; doses should be titrated to achieve an increase of 15 mmHg in mean arterial pressure. Octreotide is administered as 100–200 μg subcutaneously every 8 hours. In case of nonresponse, doses of both drugs can be increased on day 3 of treatment. In a pilot study, the combination of midodrine and octreotide, plus albumin restored kidney function in approximately 40% of patients with HRS.

Several meta-analyses have evaluated and compared the efficacy of vasoconstrictors, where studies have shown that terlipressin, in combination with IV albumin, has the highest efficacy. Although comparisons of terlipressin with norepinephrine and norepinephrine with octreotide and midodrine did not show any significant differences, terlipressin had better efficacy in reversing HRS than midodrine plus octreotide. In terms of overall mortality, meta-analyses results have revealed that most vasoconstrictors did not show any significant reduction in overall mortality. Although these results are disappointing, it must be noted that interventions to improve kidney function do not improve the underlying poor hepatic function in patients with HRS.

**Albumin**

Albumin is often administered in combination with vasoconstrictors to counteract the reduction in EABV and improve cardiac contractility. The efficacy of terlipressin when administered in combination with albumin has been proven in a large number of studies. In the only study in which terlipressin was used alone for the treatment of HRS, the efficacy of terlipressin was much lower than when it was used in combination with albumin. This may be due to the ability of albumin to maintain or increase cardiac output even in the most advanced phases of liver disease. The recommended dose is generally 20–40 g IV once daily after the initial dose of albumin is administered as 1 g/kg/day for 2 days.

Accumulating experimental and clinical evidence is suggesting that not only is albumin capable of increasing systemic vascular resistance and cardiac output, its capacity of exerting anti-oxidant and anti-inflammatory actions also plays a role in mitigating the inflammatory state associated with HRS. Albumin is able to bind a wide range of substances, including various bacterial products, bile acids, cytokines, nitric oxide, and endotoxins. Although this results in a significant reduction in serum creatinine levels in patients with HRS, in patients refractory to vasoconstrictors, improvements in kidney function and systemic hemodynamics are not observed upon administration of IV albumin, despite a reduction in NO concentrations.

**Role of kidney replacement therapy in the treatment of hepatorenal syndrome**

Although there is no definite role of KRT in the treatment of AKI in patients with cirrhosis, KRT may be indicated in those unresponsive to pharmacological treatment and with conventional indications for KRT such as volume overload, uremia, or electrolyte imbalances, as well as a bridging therapy to transplantation. According to a retrospective study that involved HRS patients who were non-responders to vasoconstrictor therapy, KRT did not provide any significant improvements in either 30-day or 180-day survival, and only led to significantly longer hospital stays. The ideal timing and the best modality of KRT has not been studied in patients with cirrhosis, and so the decision to initiate KRT should be made on clinical grounds, such as worsening kidney function with intractable volume overload, diuretic intolerance or resistance, or medically refractory electrolyte disturbances. To prevent fluid accumulation, KRT should also be considered if the daily fluid balance cannot be maintained, regardless of urine output. Continuous kidney replacement therapy (CKRT) should be used in hemodynamically unstable patients, and also has the advantage of not increase intracranial pressure, which is in contrast to conventional KRT. In cirrhotic patients with hyperammonemia and encephalopathy, CKRT may be used to mitigate cerebral edema and encephalopathy, but the cut-off ammonia level requiring initiation of CKRT is unknown.

In the setting of CKRT, the molecular adsorbent recirculating system (MARS) is a potential therapeutic modality. MARS is an extracorporeal liver support system based on albumin dialysis, given that albumin is one of the most important molecules involved in the detoxification and the liver regulation process. MARS removes albumin-bound toxins which may have detrimental effects on hepatocytes and other organs, as well as other water-soluble cytokines. A small prospective controlled trial involving 13 patients with cirrhosis and HRS demonstrated that mortality at day 7 was significantly lower than in the control group, suggesting that the removal of albumin-bound substances with the MARS method may contribute to the treatment of HRS. However, in a
larger study involving 189 patients with acute-on-chronic liver failure, although MARS significantly decreased serum creatinine levels at day 4 compared to standard medical treatment, there was no significant difference in the 28-day mortality between the two treatment groups. Given the conflicting results, further observational and prospective controlled trials are needed for the generalized application of supportive detoxification therapies.

Transjugular intrahepatic portosystemic shunt

In theory, a transjugular intrahepatic portosystemic shunt (TIPS) that connects the portal vein with one of the hepatic veins may improve kidney function in HRS by decreasing portal hypertension and reducing and reversing the hemodynamic changes that precipitate HRS. However, there is only a paucity of studies that have looked into the role of TIPS in HRS, so its use remains controversial in this population group. Although results of a few studies have demonstrated that TIPS could improve kidney function by improving serum creatinine, serum sodium, and urine output, the relative liver ischemia that immediately follows TIPS insertion could potentially precipitate hepatic failure in patients with predisposed severe liver dysfunction. Hopefully, the ongoing Liver-Hero (HRS-AKI Treatment With Tips in Patients with Cirrhosis) trial that compares the effectiveness and safety of TIPS implantation in patients with stage 2 and 3 HRS-AKI and liver cirrhosis with standard therapy of terlipressin and albumin will clarify the role of TIPS for use in this patient population.

Liver transplantation

LT remains the definitive treatment for HRS. Although simultaneous liver-kidney transplantation (SKLT) is the procedure of choice if native kidney function recovery is not expected after LT, the decision to perform SKLT versus LT remains a challenge. Predicting the recovery of impaired kidney function is challenging because various factors, particularly the duration of kidney injury, contributes to kidney prognosis. In the US, the Organ Procurement and Transplantation Network policy for simultaneous liver-kidney organ allocation requires an eGFR of ≤25 mL/min/1.73m² for 6 weeks or a period of kidney replacement therapy of ≥6 weeks in patients with AKI, presence of CKD G3b, which is defined as eGFR of <44 mL/min/1.73m² for >90 days, or comorbid presence of metabolic diseases. European guidelines recommend that patients with end-stage liver disease who also have CKD G4 or 5, defined as eGFR <30 mL/min/1.73m², or type 1 HRS requiring kidney replacement therapy of >8–12 weeks and patients with kidney biopsy samples revealing >30% glomerulosclerosis and fibrosis should receive SLKT. Nevertheless, in approximately 10% of patients who receive LT may have persistent or progressive kidney dysfunction even after a successful transplant. In particular, patients with ATN are at higher risk of CKD post-transplant, and the lack of ideal biomarkers often results in misdiagnosis.

CONCLUSIONS

There have been considerable improvements in the diagnosis and management of HRS, as well as evolving definitions, advances in pathophysiological understanding, and biomarker discovery. Lowering of the serum creatinine level threshold for the diagnosis of HRS-AKI has allowed for earlier recognition and treatment. In terms of pathophysiology, it is now recognized that HRS not only involves circulatory dysfunction, but also systemic inflammation. New insights into the pathophysiological basis have allowed for further investigation into novel therapeutic agents that target specific pathophysiological pathways. Although the differential diagnosis of AKI in patients with cirrhosis is difficult, recent studies of novel biomarkers have allowed for further investigations into tools that may assist the clinician in the diagnosis and management of HRS-AKI. Moreover, the findings from recent large-scale randomized clinical trials have further supported the use of terlipressin. While LT remains the optimal treatment option, particularly in patients with a high risk of persistent kidney dysfunction, SLKT may be warranted over LT alone. During the patient’s time on the liver transplant waiting list, CKRT may be considered as a bridging therapy to transplantation.

Future perspectives

Despite the recent advances in HRS, much remains to be uncovered. Although there have been consistent efforts into updating the definition of HRS over the past decades, the
clinical implications of the newly proposed diagnostic criteria are still unclear. Hopefully, results from future validation studies will further refine the diagnostic criteria to allow for earlier recognition and thus, management of HRS. Although it is now recognized that systemic inflammation plays an important role in the pathophysiology of HRS, the exact mechanisms by which systemic inflammation leads to HRS remain to be elucidated. While novel biomarkers that differentiate structural from functional AKI have been recently investigated, their predictive performances are far from optimal, and therefore further validation studies are needed. Not only do these novel biomarkers have the potential to differentiate AKI etiology, but they also have the potential to predict treatment response of HRS, as well as predict the prognosis of patient with HRS. Despite the positive results from several randomized clinical trials that have supported the use of terlipressin in patients with HRS-AKI, terlipressin is a drug not without significant side effects, and therefore the risk-benefit of administering terlipressin in combination with IV albumin should be carefully considered. Investigation into novel biomarkers that will not only allow for adequate selection of patients for vasoconstrictor therapy and proportional albumin use to reduce risk of adverse events, but also assist in predicting the reversibility of kidney dysfunction after LT is warranted.

Authors’ contribution
Conception and design: J.W. Chang; Writing, review, and/or revision of the manuscript: C.Y. Jung and J.W. Chang; Administrative, technical, or material support: J.W. Chang; Study supervision: J.W. Chang.

Acknowledgements
The authors thank the Medical Illustration & Design team of the Medical Research Support Services of Yonsei University College of Medicine for all artistic support related to this work.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

904


120. Wong F, Raina N, Richardson R. Molecular adsorbent recirculating system is ineffective in the management of type 1 hepatorenal syndrome in patients with cirrhosis with ascites who have failed vasoconstrictor treatment. Gut 2010;59:381-386.


The evolving role of lenvatinib at the new era of first-line hepatocellular carcinoma treatment

Landon L. Chan¹ and Stephen L. Chan¹,²

¹Department of Clinical Oncology, Prince of Wales Hospital, The Chinese University of Hong Kong; ²State Key Laboratory of Translational Oncology, Sir YK Pao Centre for Cancer, Hong Kong Cancer Institute, The Chinese University of Hong Kong, Hong Kong SAR, China

Emergence of multi-targeted kinase inhibitors (MTIs) and immune checkpoint inhibitors (ICI) have changed the landscape of management in hepatocellular carcinoma (HCC). Combination therapy involving ICI has superseded sorafenib as the first-line treatment option for advanced HCC due to their superior response rates and survival benefits based on recently published phase III trials. However, the role of first-line lenvatinib remains uncertain as no prospective trials have compared its efficacy with ICI in advanced HCC. Several retrospective studies have shown that first-line lenvatinib may not be inferior to ICI combination. Indeed, a growing body of evidence suggests that ICI treatment is associated with inferior treatment outcome in non-viral HCC patients, questioning the supremacy of ICI treatment in all patients and rendering first-line lenvatinib as a potential preferred treatment option. Furthermore, in high-burden intermediate-stage HCC, accumulating evidence supports first-line lenvatinib, or in combination with transarterial chemoembolization (TACE), as a preferred treatment option over TACE alone. In this Review, we describe the latest evidence surrounding the evolving role of first-line lenvatinib in HCC. (Clin Mol Hepatol 2023;29:909-923)

Keywords: Hepatocellular carcinoma; Antineoplastic agents; Immune checkpoint inhibitor

INTRODUCTION

Hepatocellular carcinoma (HCC) is a huge global healthcare burden according to the latest GLOBOCAN statistics.¹ In 2020, primary liver cancer (with HCC representing ~75–85% of cases) ranked the sixth most commonly diagnosed cancer with approximately 906,000 new cases, and the third leading cause of cancer mortality worldwide resulting in 830,000 deaths.¹ Despite improvement in surveillance strategies, many HCC patients present at an advanced stage where systemic therapy is a central component of treatment.

Systemic treatment has been limited for HCC. Sorafenib was the first multi-targeted kinase inhibitor (MTI) approved for the treatment of advanced HCC. It was approved in 2007 based on the SHARP trial, in which sorafenib improved progression-free survival (PFS) from 2.8 months to 5.5 months (hazard ratio [HR]: 0.58; P<0.001), and overall survival (OS) from 7.9 months to 10.7 months (HR: 0.69; P<0.001).² Despite these statistically significant findings, the objective response rate (ORR) of sorafenib was only 2%, and majority of patients treated with sorafenib achieved stable disease only (Tables 1 and 2). Unfortunately, a number of subsequent trials testing...
other MTIs had failed to demonstrate superiority compared to sorafenib, and sorafenib remained as the only systemic treatment option for advanced HCC for the next ten years.

In the recent five years, systemic treatment of HCC was met with an expansion of treatment options including both MTIs and immune checkpoint inhibitors (ICI). The approval of lenvatinib as the first-line treatment in advanced HCC in 2018 based on the noninferiority REFLECT trial marked the turning point of systemic treatment options in advanced HCC (Tables 1 and 2). Currently, OS for HCC patients with advanced disease have become more than doubled from a few months only in the era of SHARP trial to more than one and a half year in the immunotherapy era. In particular, the introduction of ICI as a treatment strategy has revolutionized the treatment paradigm of many cancers, including HCC. Combination of atezolizumab (anti-PD-L1) plus bevacizumab (anti-VEGF), or durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA4), has demonstrated unprecedented high ORR in the range of 20% to 30%, and OS in the range of 16 to 19 months unseen in the history of HCC (Tables 1 and 2). These ICI combinations have now become the recommended first-line treatment options for advanced HCC.

With these rapid developments of systemic treatment options for HCC, there is much ambiguity on the role of lenvatinib in the first-line setting. In particular, given the remarkable clinical outcomes offered by ICI combinations, should we cast away lenvatinib as a treatment option in the first-line setting in advanced disease? Alternatively, are there situations where lenvatinib may be a reasonable, or perhaps a more suitable, first-line treatment option in the management of HCC? In this Review, we will address these controversies and discuss about the evolving role of lenvatinib as a first-line treatment option for HCC.

LENVATINIB MONOTHERAPY AS FIRST-LINE TREATMENT IN ADVANCED HCC

Patients who are not suitable for ICI therapy

Lenvatinib is an oral MTI that targets the VEGF receptors 1–3, FGF receptors 1–4, PDGF receptor alpha, RET, and KIT. Lenvatinib monotherapy was approved for advanced HCC based on the REFLECT study which showed noninferiority of lenvatinib compared to sorafenib. In the trial, patients who received lenvatinib had a median OS of 13.6 months compared to 12.3 months for patients who received sorafenib (HR 0.92, 95% confidence interval [CI] 0.79–1.06). Patients treated with lenvatinib also had longer PFS (7.4 vs. 3.7 months; HR: 0.66, 95% CI 0.57–0.77) and higher ORR (24.1% vs. 9.2%; OR: 3.13, 95% CI 2.15–4.56) compared to sorafenib. Treatment emergent adverse events were similar between the two drugs. In a post-hoc analysis of patient-reported outcomes (PRO) of the REFLECT trial, most PRO scales generally favoured the lenvatinib group. Patients treated with lenvatinib experienced statistically significant delays in fatigue, pain and diarrhea compared to sorafenib. In the real world setting, the similar OS observed between sorafenib and lenvatinib have also been demonstrated in a meta-analysis including 15 studies containing 3,908 patients from both Asian and Western populations, with consistent findings of higher ORR and prolonged PFS with lenvatinib compared to sorafenib. Furthermore, lenvatinib was associated with higher incidence of asymptomatic adverse events such as hypertension, proteinuria and hypothyroidism, whereas sorafenib was associated with higher incidence of symptomatic adverse events such as palmar-plantar erythrodysesthesia and diarrhea (Table 3). Therefore, lenvatinib might be preferable over sorafenib in clinical practice if MTI monotherapy is prescribed as systemic treatment.

However, the role of MTI monotherapy as first-line treatment in advanced HCC has diminished since the introduction of ICI combinations. The current recommended first-line treatment for advanced HCC is either atezolizumab plus bevacizumab based on the IMBrave 150 trial, or durvalumab

Abbreviations:

MTIs, multi-targeted kinase inhibitors; ICI, immune checkpoint inhibitor; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; PFS, progression-free survival; HR, hazard ratio; OS, overall survival; ORR, objective response rate; AFP, alpha fetoprotein; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group

https://doi.org/10.3350/cmh.2023.0114
http://www.e-cmh.org
<table>
<thead>
<tr>
<th>Trial name</th>
<th>SHARP</th>
<th>REFLECT</th>
<th>IMBrave 150</th>
<th>HIMALAYA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug studied</td>
<td>Sorafenib</td>
<td>Lenvatinib</td>
<td>Atezolizumab and bevacizumab</td>
<td>Tremelimumab and Durvalumab</td>
</tr>
<tr>
<td>Control arm</td>
<td>Placebo</td>
<td>Sorafenib</td>
<td>Sorafenib</td>
<td>Sorafenib</td>
</tr>
<tr>
<td>Published year</td>
<td>2008</td>
<td>2018</td>
<td>2020</td>
<td>2023</td>
</tr>
<tr>
<td>Number of patients in the treatment arm</td>
<td>299</td>
<td>478</td>
<td>336</td>
<td>393</td>
</tr>
<tr>
<td>Median age</td>
<td>64.9</td>
<td>63</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>87%</td>
<td>85%</td>
<td>82%</td>
<td>83.2%</td>
</tr>
<tr>
<td>ECOG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>54%</td>
<td>63%</td>
<td>62%</td>
<td>62.1%</td>
</tr>
<tr>
<td>1</td>
<td>38%</td>
<td>37%</td>
<td>38%</td>
<td>37.7%</td>
</tr>
<tr>
<td>HCC etiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>19%</td>
<td>53%</td>
<td>49%</td>
<td>31%</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>29%</td>
<td>19%</td>
<td>21%</td>
<td>28%</td>
</tr>
<tr>
<td>Non-viral</td>
<td>51%</td>
<td>28%</td>
<td>30%</td>
<td>41%</td>
</tr>
<tr>
<td>BCLC stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>B</td>
<td>8%</td>
<td>19%</td>
<td>15%</td>
<td>19.6%</td>
</tr>
<tr>
<td>C</td>
<td>82%</td>
<td>81%</td>
<td>82%</td>
<td>80.4%</td>
</tr>
<tr>
<td>Child-Pugh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>95%</td>
<td>99%</td>
<td>100%</td>
<td>98.5%</td>
</tr>
<tr>
<td>B</td>
<td>5%</td>
<td>1%</td>
<td>0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Macroscopic Portal Vein invasion</td>
<td>36%</td>
<td>19%</td>
<td>38%</td>
<td>26.2%</td>
</tr>
<tr>
<td>Extrahepatic spread</td>
<td>53%</td>
<td>62%</td>
<td>63%</td>
<td>53.2%</td>
</tr>
<tr>
<td>Key exclusion criteria</td>
<td>-</td>
<td>Main portal vein or bile duct invasion; tumors &gt;50% of liver occupation; gastrointestinal bleeding event within 28 days prior to randomization</td>
<td>High bleeding risk; untreated gastric or esophageal varices; prior bleeding event due to esophageal or gastric varices in 6 months</td>
<td>Main portal vein thrombosis; active of prior GI variceal bleed within 12 months</td>
</tr>
</tbody>
</table>

MTIs, multi-targeted kinase inhibitors; ICI, immune checkpoint inhibitor; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; GI, gastrointestinal.
plus tremelimumab based on the HIMALAYA trial. These ICI combinations have demonstrated superior ORR and OS benefits over sorafenib. In the updated analysis of IMBrave 150, atezolizumab plus bevacizumab prolonged PFS for 2.6 months, from 4.3 months to 6.9 months, and prolonged OS for 5.8 months, from 13.4 months to 19.2 months compared to sorafenib. Higher ORR was observed in the atezolizumab plus bevacizumab group (30% and 11%) (Table 2). Incidence of grade 3 to 4 treatment-related adverse events were similar between the two treatment arms (Table 3). In the HIMALAYA trial, durvalumab plus tremelimumab was associated with improved OS at 16.4 months compared to sorafenib at 13.8 months, and a higher ORR with 20.1% for the durvalumab plus tremelimumab group compared to 5.1% for the sorafenib group. But the PFS was similar between durvalumab plus tremelimumab and sorafenib (3.8 vs. 4.1 months) (Table 2). Notably, the survival curve for patients treated with durvalumab and tremelimumab plateau at around 30%, implying a significant proportion of patients were long-term survivors. Despite the higher ORR and survival offered by ICI combinations, there are scenarios in which clinicians may consider lenvatinib over ICI combinations, considering patients’ comorbidities, physical conditions and preferences. For instance, patients with untreated or incompletely treated esophageal or gastric varices with signs of portal hypertension should avoid atezolizumab plus bevacizumab, because of the significant bleeding risk associated with high dose bevacizumab (15 mg/kg). Patients with underlying autoimmune diseases are at risk of disease flare (up to 50%) or the occurrence of other immune-related adverse events if given ICIs. Indeed, this group of patients is usually excluded from clinical trials testing ICIs. Furthermore, the management of immunosuppressive drugs at the beginning of ICI therapy in patients with pre-existing autoimmune disease remains a question in clinical practice.

Importantly, a minority group (~10–16%) of HCC patients developed disease recurrence after liver transplantation. Liver transplantation is a potential curative treatment option for selected HCC patients who fulfilled the Milan criteria. Patients with liver transplantation require long-term immunosuppressive drugs to avoid acute or chronic rejection. The use of ICIs in HCC recurrence post liver transplantation is controversial due to the risk of enhancing alloimmunity and inducing rejection, as well as concerns of the efficacy of ICIs under the background of immunosuppressants. Indeed, evidence on this topic is scarce. A recent literature review including 27 cases of liver transplants with HCC recurrence treated with ICIs, 8 (29.6%) patients had disease control, but 6 (22.2%) patients developed acute graft rejection. Therefore, the most suitable systemic therapy for HCC recurrence post liver transplant is still MTI. Sorafenib, being the MTI with the longest history in the treatment for HCC, has accumulated the highest amount of evidence in this group of patients. Recently, more evidence is also available for lenvatinib. In a retrospective case-control study in Taiwan, 10 patients were

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Studied drug</th>
<th>mPFS (mo)</th>
<th>mOS (mo)</th>
<th>ORR (%) by RECIST 1.1</th>
<th>DCR (%) by RECIST 1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHARP</td>
<td>Sorafenib</td>
<td>5.5</td>
<td>10.7</td>
<td>2.3</td>
<td>43</td>
</tr>
<tr>
<td>REFLECT</td>
<td>Lenvatinib</td>
<td>7.3</td>
<td>13.6</td>
<td>18.8</td>
<td>72.8</td>
</tr>
<tr>
<td>REFLECT</td>
<td>Sorafenib</td>
<td>3.6</td>
<td>12.3</td>
<td>6</td>
<td>59</td>
</tr>
<tr>
<td>IMBrave 150</td>
<td>Atezolizumab plus Bevacizumab</td>
<td>6.9</td>
<td>19.2</td>
<td>30</td>
<td>74</td>
</tr>
<tr>
<td>IMBrave 150</td>
<td>Sorafenib</td>
<td>4.3</td>
<td>13.4</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>HIMALAYA</td>
<td>Durvalumab plus Tremelimumab</td>
<td>3.8</td>
<td>16.4</td>
<td>20.1</td>
<td>60.1</td>
</tr>
<tr>
<td>HIMALAYA</td>
<td>Sorafenib</td>
<td>4.1</td>
<td>13.8</td>
<td>5.1</td>
<td>60.7</td>
</tr>
<tr>
<td>LEAP 002</td>
<td>Lenvatinib plus pembrolizum</td>
<td>8.2</td>
<td>21.2</td>
<td>26.1</td>
<td>-</td>
</tr>
<tr>
<td>LEAP 002</td>
<td>Lenvatinib</td>
<td>8.1</td>
<td>19.0</td>
<td>17.5</td>
<td>-</td>
</tr>
<tr>
<td>COSMIC 312</td>
<td>Cabozantinib plus Atezolizumab</td>
<td>6.8</td>
<td>15.4</td>
<td>11</td>
<td>78</td>
</tr>
<tr>
<td>COSMIC 312</td>
<td>Sorafenib</td>
<td>4.2</td>
<td>15.5</td>
<td>4</td>
<td>65</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; DCR, disease control rate; mPFS, median progression free survival; mOS, median overall survival; ORR, objective response rate.
<table>
<thead>
<tr>
<th>Study name</th>
<th>Studied drug</th>
<th>Retrospective or prospective</th>
<th>Number of patients</th>
<th>Top 3 most common toxicities</th>
<th>Grade 3 or 4 toxicity (%)</th>
<th>Top 3 most common grade 3 or 4 toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>REFLECT (2018)</td>
<td>Lenvatinib</td>
<td>Prospective</td>
<td>476</td>
<td>Hypertension (42%), diarrhea (39%), decreased appetite (34%)</td>
<td>75</td>
<td>Hypertension (23%), decreased weight (8%), increased blood bilirubin (7%)</td>
</tr>
<tr>
<td>REFLECT (2018)</td>
<td>Sorafenib</td>
<td>Prospective</td>
<td>475</td>
<td>PPES (52%), diarrhea (46%), decreased appetite (30%)</td>
<td>67</td>
<td>Hypertension (14%), PPES (11%), AST increase (8%)</td>
</tr>
<tr>
<td>IMBrave 150 (2020)</td>
<td>Atezolizumab plus bevacizumab</td>
<td>Prospective</td>
<td>329</td>
<td>Hypertension (15.2%), AST increase (7.0%), ALT increase (3.6%)</td>
<td>56.5</td>
<td>Hypertension (15.2%), AST increase (7.0%), ALT increase (3.6%)</td>
</tr>
<tr>
<td>IMBrave 150 (2020)</td>
<td>Sorafenib</td>
<td>Prospective</td>
<td>156</td>
<td>Diarrhea (49.4%), PPSE (48.1%), decreased appetite (24.4%), hypertension (24.4%)</td>
<td>55.1</td>
<td>Hypertension (12.2%), PPSE (8.6%), Blood bilirubin increase (6.4%)</td>
</tr>
<tr>
<td>HIMALAYA (2023)</td>
<td>Durvalumab plus tremelimumab</td>
<td>Prospective</td>
<td>388</td>
<td>Diarrhea (26.5%), pruritus (22.9%), rash (22.4%)</td>
<td>50.5</td>
<td>Lipase increase (6.2%), AST increase (5.2%), diarrhea (4.4%)</td>
</tr>
<tr>
<td>HIMALAYA (2023)</td>
<td>Sorafenib</td>
<td>Prospective</td>
<td>374</td>
<td>PPES (46.5%), diarrhea (44.7%), fatigue (19.0%)</td>
<td>52.4</td>
<td>PPES (9.1%), hypertension (6.1%), diarrhea (4.3%)</td>
</tr>
<tr>
<td>LEAP-002 (2022)</td>
<td>Lenvatinib</td>
<td>Prospective</td>
<td>208</td>
<td>-</td>
<td>57.5</td>
<td>-</td>
</tr>
<tr>
<td>LEAP-002 (2022)</td>
<td>Lenvatinib plus pembrolizumab</td>
<td>Prospective</td>
<td>174</td>
<td>-</td>
<td>62.5</td>
<td>-</td>
</tr>
<tr>
<td>COSMIC-312</td>
<td>Cabozantinib plus aezolizumab</td>
<td>Prospective</td>
<td>432</td>
<td>Diarrhea (44%), PPSE (34%), decreased appetite (25%)</td>
<td>64</td>
<td>AST increase (8%), ALT increase (9%), Hypertension (9%)</td>
</tr>
<tr>
<td>COSMIC-312</td>
<td>Sorafenib</td>
<td>Prospective</td>
<td>217</td>
<td>Diarrhea (45%), PPSE (36%), decreased appetite (18%)</td>
<td>46</td>
<td>PPES (8%), hypertension (8%), abdominal pain (5%)</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>Lenvatinib</td>
<td>Retrospective</td>
<td>146</td>
<td>Anorexia (28.8%), fatigue (24.7%), AST increase (24%)</td>
<td>21.9</td>
<td>Anorexia (6.8%), proteinuria (2.7%), hypertension (2.7%), diarrhea (2.7%)</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>Atezolizumab plus bevacizumab</td>
<td>Retrospective</td>
<td>86</td>
<td>Hypertension (41.9%), AST elevation (37.2%), thrombocytopenia (36.0%), fatigue (36.0%)</td>
<td>42.8</td>
<td>AST elevation (8.1%), hypertension (5.8%), total bilirubin elevation (3.5%)</td>
</tr>
<tr>
<td>Casadei-Gardini et al. (2023)</td>
<td>Lenvatinib</td>
<td>Retrospective</td>
<td>1343</td>
<td>Fatigue (32.1%), anorexia (31.8%), hypertension (31.5%)</td>
<td>68.7</td>
<td>Proteinuria (7.1%), hypertension (6.0%), anorexia (5.4%)</td>
</tr>
<tr>
<td>Casadei-Gardini et al. (2023)</td>
<td>Atezolizumab plus bevacizumab</td>
<td>Retrospective</td>
<td>864</td>
<td>Proteinuria (27.6%), hypertension (25.8%), fatigue (24.8%)</td>
<td>48.8</td>
<td>Hypertension (6.6%), proteinuria (6.1%), fatigue (1.9%)</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PPES, palmar-plantar erythrodysaesthesia syndrome; ICI, immune checkpoint inhibitor.
identified to have received lenvatinib after disease recurrence post liver transplantation. The median PFS and OS were 3.7 and 16.4 months respectively. In this small cohort of patients, 20% of patients achieved partial response and 50% of patients achieved stable disease. Adverse events were predominately grade 1 to 2, with only 1 patient developed grade 3 hypertension. Compared to the control group which were 25 HCC patients without liver transplantation who received second-line lenvatinib, there were no difference in PFS, OS or pattern of adverse events observed. In another multinational, multicenter, retrospective study evaluating 45 patients with recurrent HCC after liver transplantation, lenvatinib achieved a median PFS and OS of 7.6 months and 14.5 months respectively. The most common grade 3 adverse event was hypertension, which developed in 20% of patients. There were no grade 4 toxicity observed. In another case series conducted in Milan of 9 HCC recurrence post liver transplantation, lenvatinib was associated with a median PFS of 321 days and 1 patient experienced grade 3 adverse event (nephrotic syndrome) requiring drug withdrawal. Comparing with a matched cohort of patients treated with sorafenib, lenvatinib was associated with a better median PFS and OS. Overall, lenvatinib is also an effective treatment option for recurrent HCC post liver transplantation, with no new toxicity signal seen.

Patients with severe portal hypertension or main portal vein thrombosis

Patients with severe portal hypertension or main portal vein thrombosis (Vp4) represent a group with particularly poor prognosis and at high risk of treatment-related adverse events. Extra considerations are needed when choosing systemic therapy for them. Severe portal hypertension is associated with high risk of variceal bleeding. Surveillance with endoscopy or prophylactic treatment with beta-blocker is advocated in the latest Baveno VII consensus. The use of agents with anti-VEGF properties such as lenvatinib and bevacizumab in patients with severe portal hypertension has raised concerns of increased variceal bleeding risk and mortality secondary to exacerbation of portal hypertension. In a prospective cohort study on the portal hemodynamic effects of lenvatinib in 28 advanced HCC patients, lenvatinib reduced the portal venous flow velocity, increased congestion index, and aggravated portal hypertension after 2 weeks of administration. However, bleeding events related to portal hypertension with the use of MTIs (including lenvatinib) were consistently reported to be lower than 2% in the recent published phase 3 trials. In a prospective multicenter study of 93 patients treated with lenvatinib, in which 37 patients had advance portal hypertension, OS did not seem to be compromised by advanced portal hypertension. On the contrary, the risk of variceal bleeding was elevated in IMbrave 150 at 2.4% in the atezolizumab plus bevacizumab group compared to 0.6% in the sorafenib group. Of note, this was a group of well-selected patients benefited from optimal portal hypertension prophylaxis and patients with bleeding esophageal or gastric varices have been excluded from the study already. In unscreened patients, the use of bevacizumab has been associated with 10% risk of bleeding varices based on systematic review on phase II trials.

Patients with main portal vein thrombosis (Vp4) were excluded from both the REFLECT and HIMALAYA trial. A retrospective study of 20 patients with Vp4 advanced HCC demonstrated efficacy and safety of lenvatinib, with ORR of 20% by mRECIST criteria and median OS of 6.7 months. Variceal bleed was seen in 2 (10%) patients. In another retrospective study included 41 HCC patients with major portal vein tumor thrombosis (Vp3/4) treated with sorafenib or lenvatinib, lenvatinib treatment was the only significant predictor of better OS (HR 0.19, 95% CI 0.06–0.68; P = 0.0106) and time to tumour progression (HR 0.16, 95% CI 0.05–0.56; P = 0.004). Worsening of liver function was noted in the first 2 weeks in the lenvatinib group but improved afterwards. The study did not report any incidence of variceal bleeding in the adverse events. On the contrary, in an exploratory analysis of IMbrave 150 evaluating the efficacy and safety of atezolizumab plus bevacizumab in patients with Vp4 portal vein invasion, OS was numerically higher in the atezolizumab plus bevacizumab group compared to the sorafenib group (7.6 vs. 5.5 months; HR 0.62; 95% CI 0.34–1.11) but the incidence of variceal bleeding was higher with atezolizumab plus bevacizumab group (13.6% vs. 0%).

Therefore, for patients with severe portal hypertension, durvalumab plus tremelimumab may be considered as first-line treatment as it has the least bleeding risk compared to atezolizumab plus bevacizumab and lenvatinib. Unfortunately, despite tremelimumab has been approved for use in combination with durvalumab by the Food and Drug Administration, and recently by the European Medicines Agency, it is
still under regulatory review in many places worldwide (e.g., United Kingdom, Australia, Hong Kong). The cost of tremelimumab is also prohibitive and so it is not accessible to many patients. Between atezolizumab plus bevacizumab and lenvatinib, lenvatinib is preferred if timely pre-treatment screening for variceal bleeding is not available. Similarly, for patients with main portal vein thrombosis, given the finding of 13.4% of patients developed variceal bleeding with atezolizumab plus bevacizumab in the exploratory analysis of IMBrave 150, lenvatinib might be considered safer if a timely screening of esophageal/gastric varices is not available.

Is lenvatinib inferior to ICI combination?

The current recommendation of ICI combinations as first-line treatment in advanced HCC is based on their superior response rates and survival compared to sorafenib. There is no prospective data to compare lenvatinib with ICI combinations. In fact, it is logical to expect lenvatinib to be inferior to ICI combination as lenvatinib was shown to be noninferior to sorafenib based on the REFLECT trial. Recently, evidence has emerged to suggest that first-line lenvatinib may not be inferior to first-line ICI combination. In a retrospective study including 232 advanced HCC patients conducted in three academic hospitals in Korea, treatment with either lenvatinib or atezolizumab plus bevacizumab did not result in any statistically significant difference in ORR (32.6% vs. 31.5%, \( P=0.868 \)), PFS (5.7 vs. 6.0 months; \( P=0.738 \)) and OS (not reached vs. 12.8 months; \( P=0.357 \)). Subgroup analyses showed that OS was comparable between the atezolizumab plus bevacizumab and lenvatinib group according to all strata (e.g., age, sex, performance status, etiology etc.) except for alpha fetoprotein (AFP) level, of which AFP<200 was associated with favourable outcome with lenvatinib. In terms of toxicity, more grade 3 or 4 adverse events were observed in the atezolizumab plus bevacizumab and lenvatinib group according to all strata (e.g., age, sex, performance status, etiology etc.) except for alpha fetoprotein (AFP) level, of which AFP<200 was associated with favourable outcome with lenvatinib. In terms of toxicity, more grade 3 or 4 adverse events were observed in the atezolizumab plus bevacizumab and lenvatinib group, but the difference was not statistically significant (42.8 vs. 21.9%; \( P=0.141 \)). In another large international retrospective study including 2,205 patients with advanced HCC, after balancing clinical features using inverse probability of treatment weighting methodology, there was no difference in either time to progression (HR 0.82; \( P=0.117 \)) or OS (HR 0.97; \( P=0.739 \)) comparing atezolizumab plus bevacizumab with lenvatinib. But grade 3 or 4 adverse events were more common in the lenvatinib group compared to the atezolizumab plus bevacizumab group (84.9% vs. 69.8%; \( P=0.009 \)). These retrospective results might somehow appear puzzling as one would expect lenvatinib to be inferior to ICI combination. In order to interpret these results, a few points should be considered. First, while the primary endpoint was noninferiority in OS in the REFLECT trial, there was a trend towards more favourable outcomes with lenvatinib compared to sorafenib, in terms of OS, PFS and ORR. In fact, several real-world studies have shown that lenvatinib performed much better in clinical practice than in randomized clinical trials. It has been consistently shown that lenvatinib not only offered superior ORR but also survival compared to sorafenib.

Second, evidence suggested that improved clinical outcomes were linked to more experience in management of adverse events with sorafenib. Since sorafenib and lenvatinib belonged to the same drug class and shared many pharmaceutical characteristics, it is plausible that prior experiences with sorafenib resulted in shorter learning curve in managing adverse events during lenvatinib treatment leading to better clinical outcome. Third, it was noted that more patients who had first-line lenvatinib in both retrospective studies cited above received locoregional therapy as a subsequent treatment. However, as the authors pointed out, this discrepancy could be related to the earlier approval of lenvatinib compared to atezolizumab plus bevacizumab, leading to a lack of effective second-line treatment (i.e., immunotherapy) in the post-lenvatinib setting. All in all, although many plausible hypotheses exist to explain the similar OS between patients treated with lenvatinib and atezolizumab plus bevacizumab in the real-world setting, one should keep in mind of the limitations of these retrospective studies that they were intrinsically biased and the population studied in the different treatment arms could be unbalanced. Future prospective studies with well-balanced populations comparing lenvatinib and atezolizumab plus bevacizumab, or other ICI combinations, would be needed to understand whether these treatments are indeed similar in efficacy.

In the prospective setting, lenvatinib monotherapy has also been compared to ICI combination. In the LEAP 002 trial, which was a global, randomized, double-blind, phase 3 study evaluating the efficacy and safety of lenvatinib plus pembrolizumab versus lenvatinib in the first-line setting for advanced HCC. This is the first phase 3 study of lenvatinib since the REFLECT study. Lenvatinib plus pembrolizumab failed to demonstrate improved PFS and OS according to the pre-
specified statistical significance. Combination of lenvatinib plus pembrolizumab resulted in PFS and OS of 8.2 months and 21.2 months respectively, compared to 8.1 months (HR for PFS: 0.87, 95% CI 0.73–1.02; P=0.047) and 19.0 months (HR for OS: 0.84, 95% CI 0.71–1.00; P=0.0227) respectively with lenvatinib monotherapy. Of note, the lenvatinib arm performed exceptionally well compared to the REFLECT trial (median OS 13.6 months) which included patients of similar characteristics. ORR was improved with lenvatinib plus pembrolizumab at 26.1% as compared to the lenvatinib arm at 17.5%, which was similar to the reported figures in the REFLECT trial. One major reason for the exceptional performance of lenvatinib arm was the availability of second-line treatment. In the LEAP 002 trial, 52.1% of patients on the lenvatinib arm received additional treatment, which was higher than in the REFLECT study at 33% only. Of that 52%, 22.8% received additional immunotherapy (e.g., atezolizumab plus bevacizumab), which is considered very active in HCC. In terms of toxicity, ICI combination was associated with higher toxicity, with grade 3 to 5 treatment related adverse events of 62.5% in the lenvatinib plus pembrolizumab group, compared to 57.5% in the lenvatinib group.

Taken together, it appears that first-line lenvatinib may be non-inferior to ICI combinations. In terms of toxicity, rate of grade 3 or higher toxicity was variable for lenvatinib in published studies, ranging from 20 to 75% (Table 3). In comparison, grade 3 or higher toxicity for ICI combinations was more consistently reported at around 40 to 50% (Table 3). Most common grade 3 or higher adverse events for lenvatinib was hypertension, which could usually be managed with anti-hypertensives, interruptions, and dose reductions. For ICI combinations, the type of grade 3 or higher adverse events was more variable depending on the ICI being used. Nonetheless, first-line lenvatinib monotherapy may allow for titration of dose according to patients’ performance status and tolerability, which could be a more versatile treatment option for those with borderline fitness to systemic treatment. Indeed, a recent retrospective study including 176 patients with advanced HCC treated with lenvatinib showed that upfront dose reduction of lenvatinib was not associated with inferior survival outcome.

Does etiology of HCC have an impact on treatment outcome?

In the past in which treatment for advanced HCC was mainly MTIs, it was thought that the etiology of HCC did not have an impact on HCC. However, after the introduction of ICI in the management of advanced HCC, evidence is accumulating that the etiology of HCC might have an impact on treatment outcomes. For example, in the updated analysis of IMBrave 150, atezolizumab plus bevacizumab resulted in improved PFS and OS compared to sorafenib, in hepatitis B HCC (HR for OS: 0.58, 95% CI 0.40–0.83; HR for PFS: 0.51, 95% CI 0.37–0.70) but not in non-viral HCC (HR for OS: 1.05, 95% CI 0.68–1.63; HR for PFS: 0.80, 95% CI 0.55–1.17). The COSMIC 312 trial was a multi-centre, randomized, phase III trial comparing cabozantinib plus atezolizumab with sorafenib in advanced HCC. Although the trial was negative for its primary endpoint in OS, in the prespecified exploratory subgroup analysis, PFS and OS were longer with the combination treatment versus sorafenib in the hepatitis B HCC subgroup (PFS: HR 0.46, 95% CI 0.29–0.73; OS: HR 0.53, 95% CI 0.33–0.87) but not in the non-viral subgroup (PFS: HR 0.92, 95% 0.60–1.41; OS: 1.18, 95% CI 0.78–1.79). A recent translational study indicated that the use of anti-PD-1 treatment may paradoxically induces and accelerates carcinogenesis in HCC patients with underlying non-alcoholic steatohepatitis (NASH). The group found that CD8+PD1+ T-cells were specifically enriched in NASH-HCC both in mouse model and human tumours. Notably, anti-PD-1 treatment promoted tissue damage, resulted in malignant changes, and induced more aggressive behaviour of existing NASH-HCC. Furthermore, in a meta-analysis of 3 published phase III trials (CheckMate 459, Keynote 240, and IMBrave 150) conducted by the same group, they found that patients with non-viral HCC did not derive survival benefits from immunotherapy (HR 0.92, 95% CI 0.77–1.11). Instead, OS was prolonged with immunotherapy in patients with viral HCC (HR 0.64, 95% CI 0.48–0.94).

In light of these interesting data, research has grown to test if HCC patients with non-viral etiology would benefit less from immunotherapy compared to MTIs. In a recently published, multinational, prospectively consecutively enrolled, retrospective study of 759 advanced HCC with non-viral etiology, treatment with lenvatinib was associated with better OS (HR: 0.65, 95% CI 0.44–0.95; P=0.0268) and PFS (HR: 0.67, 95% CI 0.51–0.86; P=0.002) compared to atezolizumab plus
bevacizumab. In particular, in the non-alcoholic fatty liver disease (NAFLD)/NASH population, multivariate analysis showed that lenvatinib treatment was associated with longer OS (HR: 0.46, 95% CI 0.26–0.84; \(P=0.011\)) and PFS (HR: 0.55, 95% CI 0.38–0.82; \(P=0.031\)) compared to atezolizumab plus bevacizumab, but not in the non-NAFLD/NASH patient subgroup.

Several factors should be considered before using etiology of HCC to determine the first-line choice of treatment. First, it is evident that non-viral subgroup of HCC is a heterogeneous population, including patients with NAFLD, chronic alcoholism, occult HBV infection (anti-HBc positive but HBsAg negative) or patients with mixed picture from above causes. Analyses of benefits of systemic therapy in each of the above subgroup is required to understand the benefits of each systemic therapy. Second, current supporting data came from subgroup analyses of clinical trials or retrospective series, which was prone to bias. The hypothesis requires validation by prospective clinical trials comparing lenvatinib to ICI-based treatment for HCC of specific etiology subgroup. Third, more informed definitions of non-viral HCC are required as these subgroups were not clearly defined in the reported analyses. For example, the gold standard of diagnosis of NAFLD is based on histological presence of steatosis in >5% hepatocytes which can only be obtained by invasive procedures such as liver biopsy. Although non-invasive diagnosis is feasible with computed tomography and ultrasonography, the reporting of radiological images is limited by intra-observers discrepancy and the sensitivity of detection by these imaging modalities. Furthermore, concurrent fatty liver disease with viral hepatitis can occur in a high proportion of viral hepatitis patients in the current metabolic liver disease pandemic. For example, in one retrospective cohort study in Hong Kong including 270 HBV-infected patients, histologically confirmed concurrent fatty liver disease was found in 107 (39.6%) patients. Therefore, future trials should clearly define the different etiologies of HCC, and take into account the possibility of concomitant etiologies occurring in the same patient.

**First-line lenvatinib with transarterial chemoembolization (TACE)**

In addition to first-line lenvatinib monotherapy in advanced HCC, lenvatinib has also been explored in combination with TACE in advanced HCC setting to improve clinical outcome. Insufficient intrahepatic tumour response remains a major problem with repeated TACE. Upregulation of VEGF and other pro-angiogenic factors post TACE induced by the creation of ischemic tumour environment has been implicated as the major mechanism of resistance to treatment. Lenvatinib as a potent anti-angiogenic agent could theoretically offer synergism with TACE by inhibiting angiogenesis and tumour growth after TACE.

In the LAUNCH study, 338 Chinese patients with primary treatment-naive or initial recurrent advanced HCC after surgery were randomly assigned to lenvatinib or lenvatinib plus on-demand TACE (LEN-TACE). Majority of patients (>85%) had hepatitis B. TACE was given 1 day after oral administration of lenvatinib and then repeated if there were incomplete necrosis or tumour regrowth. After a median follow-up of 17 months, it was shown that the OS was significantly longer in the LEN-TACE group at 17.8 months compared to 11.8 months in the lenvatinib monotherapy group (HR: 0.45; \(P<0.001\)). The median PFS was also prolonged in the LEN-TACE group at 10.6 months compared to 6.4 months in the lenvatinib monotherapy group (HR: 0.43; \(P<0.001\)). ORR was higher in the LEN-TACE group at 54.1% compared to lenvatinib monotherapy group at 25.0% (\(P<0.001\)) by the mRECIST criteria. In terms of safety, more grade 3 or 4 deranged liver enzymes were seen in the LEN-TACE group compared to lenvatinib monotherapy group (~20% vs. 2%), but the frequency of other grade 3 or 4 adverse events such as hand-foot skin reaction, diarrhea, abdominal pain etc. were similar between the two groups. While this study showed promising evidence of first-line lenvatinib combined with TACE in hepatitis B HCC patients of Chinese ethnicity, further studies will be needed to extend this finding to HCC patients with other etiologies and ethnicities.

**LENVATINIB AS FIRST-LINE TREATMENT OPTION IN INTERMEDIATE-STAGE HCC**

Intermediate-stage HCC represents the most heterogeneous group of patients. Up until 2018, the recommended treatment for intermediate-stage (i.e., Barcelona Clinic Liver Cancer [BCLC]-B) HCC was TACE only. In the 2022 updated version, it was decided that intermediate-stage HCC should be divided into three subgroups according to tumour burden.
and liver function, to better stratify this heterogeneous patient group and guide treatment.\(^61\) In the subgroup with diffuse, infiltrative, extensive bilobar liver involvement, the recommended treatment is no longer TACE but systemic treatment. TACE is not an effective treatment strategy for high-burden intermediate-stage HCC and can lead to early liver function deterioration.\(^61,62\)

Indeed, systemic therapy for intermediate-stage HCC is nothing new. Sorafenib has been shown to be effective in intermediate-stage HCC in three large-scale real-world studies.\(^63-65\) The GIDEON trial was a global prospective observational study performed between 2009 and 2012 to evaluate the safety and efficacy of sorafenib in HCC patients at different BCLC stages. It showed that the median OS was much longer in BCLC-B patients than in BCLC-C patients (OS: 29.5 vs. 11.1 months).\(^63\) The SOFIA and INSIGHT trial, two similar studies conducted in Europe over similar period, also showed better median OS in BCLC-B patients than in BCLC-C patients when treated with sorafenib (SOFA, OS: 20.6 vs. 8.4 months; INSIGHT, OS: 19.6 vs. 13.6 months).\(^64,65\)

But which group of patients would benefit from systemic treatment instead of TACE was still largely unknown. To characterize the group of patients who would be better suited for systemic therapy, lenvatinib was evaluated against TACE as first-line treatment for intermediate-stage, TACE-naïve HCC with ‘up-to-7’ out tumor burden and Child-Pugh A liver function.\(^66\) Lenvatinib was chosen over sorafenib by virtue of its higher ORR in the REFLECT trial.\(^67,68\) The ‘up-to-7’ criteria refers to the sum of the number of lesions and the diameters of these lesions being seven or smaller. This was a criteria first developed in extension to the Milan criteria to predict outcomes for liver transplantation.\(^68\) In a proof-of-concept retrospective propensity score-matched study, it was shown that lenvatinib was associated with significantly improved OS (37.9 vs. 21.3 months; \(P<0.01\)), PFS (16.0 vs. 3.0 months; \(P<0.001\)) and ORR (73.3% vs. 33.3%; \(P<0.001\)). The study also showed that hepatic function deteriorated with repeated TACE (baseline ALBI score from –2.66 to –2.09; \(P<0.001\)) but was maintained in the group treated with lenvatinib (baseline ALBI score from –2.61 to –2.61; \(P=0.254\)). Of note, two patients achieved significant downstaging with lenvatinib enabling subsequent ablation and resection. These encouraging results warrant confirmation of the role of lenvatinib in intermediate-stage HCC with beyond ‘up-to-7’ tumour burden and preserved liver function in a large randomized controlled trial. Nonetheless, those who were thought to be poor responder of TACE should also be considered for lenvatinib up-front.

The TACTICS-L study was a Japanese, phase II, single-arm study to evaluate the efficacy and safety of combination therapy with lenvatinib and TACE in unresectable, intermediate-stage HCC.\(^69\) The study recruited 62 patients who were predominantly of advanced age (≥65 years old: 79%) with BCLC-B stage (59.7%) disease. 64.5% of patients had tumour within up-to-7 criteria. Lenvatinib was given 14 to 21 days then stopped 2 days before TACE and resumed 2 days after, until disease progression. With a median follow-up of 20.3 months, the median PFS was 28.3 months and 2-year PFS was more than 60%. ORR at best response was 88.7% with complete response observed in 66.1% of patients. Around half (50.5%) of the treatment responders (n=55) had sustained response at 1 year. This treatment approach was well tolerated with the most common adverse events being hypothyroidism (58.1%), hypertension (53.2%) and decreased appetite (50.0%). No new safety signal was observed.\(^59\) Therefore, lenvatinib-TACE is another promising first-line strategy for patients with unresectable intermediate-stage HCC despite the study recruited a significant proportion of earlier stage, BCLC-A HCC patients. Further phase III studies would be required to validate this combination approach.

On a different note, a number of phase III trials are ongoing testing the efficacy of atezolizumab plus bevacizumab (or in combination with TACE) versus TACE alone in intermediate-stage HCC (NCT04803994, NCG04712643).\(^70,71\) As the pattern of response differs between patients treated with atezolizumab plus bevacizumab (i.e., induces tumour shrinkage) and lenvatinib (i.e., induces tumour necrosis via reduced blood through),\(^72\) it would be interesting to compare atezolizumab plus bevacizumab-TACE with lenvatinib-TACE in intermediate-stage HCC in the future.

**CONCLUDING REMARKS**

In the era of effective ICI combination therapy with remarkable response rate and survival, the role of first-line lenvatinib in advanced HCC has diminished. However, not all patients are suitable for ICI therapy due to their underlying medical conditions such as autoimmune disease or on long-term immunosuppressants (Table 2). Lenvatinib in these set-
tings plays an important role and appears to be safe and equally effective. Nevertheless, clinicians should pay attention to the frequent adverse events such as hypertension, proteinuria and hypothyroidism following long-term use of lenvatinib, and it is important to manage these side effects well. In addition, with the increasing number of drugs available for the treatment of advanced HCC, the correct sequence of treatment (e.g., ICI first vs. TKI first) is currently an active area of research. Several retrospective studies have reported efficacy and safety of lenvatinib in the second-line setting post ICI but prospective data is still lacking.73,74

On a different note, we are now starting to understand that patients with HCC of different etiologies may respond to ICI therapies differently, in which some patients may achieve better response with lenvatinib. For example, multiple retrospective analyses have shown that lenvatinib might be more effective than ICI combination in non-viral HCC patients.34,53 This differential response has been attributed to the differences in tumour microenvironments and immune milieu associated with the underlying etiologies.52 Nonetheless, non-viral HCC is a heterogeneous group of patients, and future studies specifically designed for HCC patients with a specific underlying etiology will be needed to validate these postulations (Table 2).

In addition, first-line lenvatinib has now been evaluated in the intermediate-stage setting, in particular for those patients with high tumour burden, such as beyond the ‘up-to-7’ criteria (Table 2). This group of patients is known to be refractory to conventional treatment like TACE. Several studies involving small number of patients have demonstrated that lenvatinib monotherapy or in combination with TACE is effective and safe for this group of patients, with the additional benefit of preservation of liver function.66,69 In the neoadjuvant and adjuvant setting, a number of trials are ongoing exploring lenvatinib in combination with immunotherapy and/or locoregional treatments (e.g., TACE, RFA) which are expected to report outcomes in the next few years (NCT05185739, NCT04227808, NCT05113186). Combination of lenvatinib, pembrolizumab and TACE is also currently being explored in the phase III LEAP 012 study.75 Therefore, the role of lenvatinib continues to evolve in the management of HCC and will remain an important pharmaceutical agent in the years to come.

Authors’ contribution
Both authors contribute equally to the conceptualization, literature review, drafting and final review of this manuscript.

Conflicts of Interest
S.L. Chan is the advisory for Astra-Zeneca, MSD, Eisai, BMS and Roche. S.L. Chan received research fund from MSD, Bayer, Eisai, Ipsen and SIRTEX. S.L. Chan received Honoraria from Bayer, Astra-Zeneca, Eisai, Roche and MSD. S.L. Chan is the speaker for MSD, BMC, Astra-Zeneca, Eisai, Roche, Ipsen, SIRTEX and Hutchmed. L.L. Chan has received travel support from Roche.

REFERENCES


52. Pfister D, Núñez NG, Pinyol R, Govaere O, Pinter M, Szylowska M, et al. NASH limits anti-tumour surveillance in immunothera-
75. Llovet JM, Vogel A, Madoff DC, Finn RS, Ogasawara S, Ren Z, et al. Randomized phase 3 LEAP-012 study: Transcatheter chemo-
embolization with or without lenvatinib plus pembrolizumab for intermediate-stage hepatocellular carcinoma not amenable to curative treatment. Cardiovasc Intervent Radiol 2022;45:405-412.
Evidence-based hyponatremia management in liver disease

Ji Young Ryu¹, Seon Ha Baek¹, and Sejoong Kim²,³,⁴

¹Department of Internal Medicine, Hallym University Dongtan Sacred Heart Hospital, Hwaseong; ²Department of Internal Medicine, Seoul University Bundang Hospital, Seongnam; ³Center for Artificial Intelligence in Healthcare, Seoul University Bundang Hospital, Seongnam; ⁴Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Hyponatremia is primarily a water balance disorder associated with high morbidity and mortality. The pathophysiological mechanisms behind hyponatremia are multifactorial, and diagnosing and treating this disorder remains challenging. In this review, the classification, pathogenesis, and step-by-step management approaches for hyponatremia in patients with liver disease are described based on recent evidence. We summarize the five sequential steps of the traditional diagnostic approach: 1) confirm true hypotonic hyponatremia, 2) assess the severity of hyponatremia symptoms, 3) measure urine osmolality, 4) classify hyponatremia based on the urine sodium concentration and extracellular fluid status, and 5) rule out any coexisting endocrine disorder and renal failure. Distinct treatment strategies for hyponatremia in liver disease should be applied according to the symptoms, duration, and etiology of disease. Symptomatic hyponatremia requires immediate correction with 3% saline. Asymptomatic chronic hyponatremia in liver disease is prevalent and treatment plans should be individualized based on diagnosis. Treatment options for correcting hyponatremia in advanced liver disease may include water restriction; hypokalemia correction; and administration of vasopressin antagonists, albumin, and 3% saline. Safety concerns for patients with liver disease include a higher risk of osmotic demyelination syndrome.

(Clin Mol Hepatol 2023;29:924-944)

Keywords: Hyponatremia; Liver cirrhosis; Liver disease; Water-electrolyte imbalance

INTRODUCTION

Hyponatremia is the most common electrolyte disturbance encountered in clinical practice and is associated with increased mortality and morbidity rates.¹ Liver disease, especially cirrhosis, is a relatively frequent etiology of hyponatremia. Hyponatremia in liver cirrhosis is typically defined as a serum sodium (sNa) concentration <135 mmol/L; however, some experts have defined hyponatremia as an sNa concentration <130 mmol/L.²,³ The prevalence of sNa concentrations <135 mmol/L, <130 mmol/L, <125 mmol/L, and <120 mmol/L are 49.4%, 21.6%, 5.74%, and 1.2% in patients with liver cirrhosis.
Hyponatremia management in liver disease

Several studies have confirmed that hyponatremia in advanced liver cirrhosis is associated with poor outcomes, including refractory ascites (RA), hepatic encephalopathy (HE), hepatorenal syndrome (HRS), and mortality in advanced liver disease.\(^4\)\(^6\) Given that sNa levels have been integrated into the model for the end-stage liver disease (MELD) score, which is used to determine liver transplantation (LT) priority, hyponatremia management is crucial.\(^7\)

Herein, we review the causes, factors, clinical features, and pathophysiology of hyponatremia in patients with liver disease. We also discuss the general treatment for hyponatremia in this patient population and management of hyponatremia before LT.

**PATHOPHYSIOLOGY OF HYPOTONIC HYPONATREMIA IN LIVER DISEASE**

The mechanisms of hyponatremia are mainly cirrhosis-induced hemodynamic compromise and other pathogenetic or superimposed factors.

**Hypervolemic hyponatremia in patients with cirrhosis (primary mechanism) (Fig. 1)**

**Systemic or splanchnic vasodilation**

The primary pathophysiologic mechanisms that lead to hypervolemic hyponatremia in patients with cirrhosis are systemic vasodilation and arterial underfilling.\(^8\) Hyperdynamic circulation, resulting in increased cardiac output, which markedly reduces systemic vascular resistance and decreases mean arterial pressure, is common among patients with cirrhosis and progressive portal hypertension.\(^14\) As pressure is reduced due to a decrease in the effective circulation volume, sodium retention mechanisms, such as the renin-angiotensin-aldosterone system, sympathetic nervous system, and ADH, are activated to retain sodium and water.\(^16\)\(^19\) Although these factors increase extracellular sodium stores, plasma volume, and cardiac output, the net effect is sodium and water reabsorption by the kidneys because the patient is substantially depleted of body fluid.\(^15\)\(^20\) Serum osmolality is generally low in patients with decompensated cirrhosis; thus, ADH must be suppressed by osmotic stimulation.\(^15\) However, non-osmotic stimuli act predominantly over osmotic stimuli to cause hyponatremia in patients with decompensated liver cirrhosis.\(^14\)\(^15\) ADH released in response to hypovolemia in liver cirrhosis increases water permeability in the collecting ducts of the kidneys via aquaporin 2, a water channel protein.\(^21\)\(^26\) This is one of the mechanisms leading to hypervolemic, dilutional hyponatremia in liver cirrhosis.\(^14\)\(^15\)

**Other causes of hypotonic hyponatremia in patients with liver disease**

**Hypervolemic hyponatremia**

Kidney failure related to end-stage liver disease or acute...

**Abbreviations:**

ADH, antidiuretic hormone; ECF, extracellular fluid; HF, heart failure; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; LT, liver transplantation; MELD, model for end-stage liver disease; ODS, osmotic demyelination syndrome; RA, refractory ascites; SIAD, syndrome of inappropriate antidiuretics; sNa, serum sodium
Figure 1. Pathogenesis of hypervolemic hyponatremia in patients with liver cirrhosis. ADH, antidiuretic hormone; DNA, deoxyribonucleic acid; NO, nitric oxide; PG, prostaglandin; RES, reticuloendothelial system.
kidney injury, including HRS, impairs urine dilution and causes dilutional hyponatremia accompanied by water retention.\textsuperscript{1,27-29} Nephrotic syndrome due to hepatitis B or C viral infection causes hyponatremia.\textsuperscript{27,30,31} Coexistent cardiac disorders, including heart failure (HF) or cardiomyopathy (e.g., alcoholic or hemochromatosis) in patients with liver disease, may result in hyponatremia via neurohormonal mechanisms.\textsuperscript{27,12}

**Hypovolemic hyponatremia**
Depletion of effective arterial volume via non-renal sodium loss (lactulose-associated diarrhea or vomiting), renal sodium loss (diuretics such as spironolactone/epiurenone), or primary adrenal insufficiency (autoimmune adrenalitis in autoimmune hepatitis or adrenal tuberculosis in alcoholics) can markedly increase secondary ADH release leading to water retention.\textsuperscript{1,27,33}

**Euvolemic hyponatremia**
Syndrome of inappropriate antidiuresis (SIAD) may be caused by infection or medications used in the treatment of liver disease (terlipressin,\textsuperscript{34-36} interferon, brivanib,\textsuperscript{37} sorafenib,\textsuperscript{37} cixutumumab,\textsuperscript{38} and boceprevir\textsuperscript{39}), acute intermittent porphyria, and malignancy (hepatocellular carcinoma).\textsuperscript{27} Endocrine disorders, including secondary adrenal insufficiency (caused by the pituitary gland or hypothalamus) or severe hypothyroidism, are rare causes of hyponatremia in patients with liver disease.

**CLASSIFICATION OF HYPONATREMIA**

The accurate classification of hyponatremia in patients with liver disease is crucial for appropriate diagnosis and management. Hyponatremia is commonly classified based on the sNa concentration, severity of clinical symptoms, duration, and extracellular fluid (ECF) status (Table 1).

**Classification of hyponatremia based on the severity of clinical symptoms and duration**
According to the severity of clinical symptoms, hyponatremia can be divided into either moderate (nausea, confusion, and headache) or severe/profound (vomiting, seizure, cardiopulmonary collapse, and coma). Based on the duration, hyponatremia can be differentiated as acute (<48 h) or chronic (>48 h).\textsuperscript{1} Patients with acute hyponatremia develop neurological symptoms due to cerebral edema because they do not have enough time to develop mechanisms that can prevent cerebral edema.\textsuperscript{40} However, most patients with advanced cirrhosis have chronic hyponatremia, which allows for astrocytes to adapt and thus symptoms may not appear, making a diagnosis based on symptoms difficult.\textsuperscript{15}

**Classification of hyponatremia based on volume status**
Hyponatremia is classified into three types according to their ECF status: hypervolemic, euvolemic, and hypovolemic hyponatremia.\textsuperscript{41-43} Patients with liver cirrhosis commonly have hypervolemia (increased ECF volume), characterized by ascites, anasarca, and/or pedal edema.\textsuperscript{2,15,20} Hypervolemia is caused by sodium retention with impaired free-water excretion, resulting from renin-angiotensin-aldosterone or sympathetic nervous system activity and ADH hypersecretion, leading to dilutional hyponatremia.\textsuperscript{2,15} Euvolemic hyponatremia is rare except when with SIAD or endocrine disorders, including adrenal insufficiency and severe hypothyroidism. Some patients (<10%) with liver cirrhosis have hypovolemic hyponatremia due to ECF loss after diuretic use or diarrhea/vomiting, characterized by a lack of ascites and pedal edema.\textsuperscript{44}

**DIAGNOSTIC APPROACH TO HYPONATREMIA**

Based on a comprehensive assessment of the history (recent body fluid loss, high water intake with low salt, presence of a malignant tumor, recent surgery, and use of drugs [thiazide diuretics, intravenous immune globulin, and terlipressin], physical examination (detailed neurologic evaluation and assessment of ECF volume), and laboratory findings (serum and urine osmolality and urine sodium), the physician can determine the underlying cause of hyponatremia and adjust the treatment accordingly for patients with liver disease.\textsuperscript{45-47}

**Steps for hyponatremia diagnosis**
A practical diagnostic approach to hyponatremia, which is similar to that used for the general population, is utilized for
patients with liver disease. The step-by-step process is presented below (Fig. 2).

**Step 1. Rule out pseudohyponatremia and calculate the glucose-corrected sNa concentration in patients with hyperglycemia**

The main objective of this step is to determine whether the hyponatremia is hypotonic. The serum osmolality is measured to differentiate hypotonicity. For serum osmolality that is <275 mOsm/kg (hypotonic hyponatremia), further predefined steps are followed, while for serum osmolality that is ≥275 mOsm/kg, hyponatremia may be hypertonic (usually from hyperglycemia) or isotonic (due to pseudohyponatremia). In patients with hyperglycemia, the corrected sNa concentrations are calculated based on equations (1) and (2).

Hillier et al., 1999: corrected Na level=Na+0.024×(serum glucose [mg/dL]−100) \( (1) \)

Katz, 1973: corrected Na level=Na+0.016×(serum glucose [mg/dL]−100) \( (2) \)

Pseudohyponatremia is defined as a spurious decrease in plasma sodium (<135 mmol/L) due to marked hyperproteinemia or hyperlipidemia. Hyperproteinemia with increased globulin due to autoimmune hepatitis (elevated immunoglobulin G), primary biliary cirrhosis, alcoholic liver disease (elevated immunoglobulin M and immunoglobulin A), or liver cirrhosis (elevated γ-globulin) are occasionally observed in patients with chronic liver disease. Moreover, marked dyslipidemia is often observed in patients with liver disease. Hypertriglyceridemia can occur due to excessive alcohol consumption, interferon treatment, and uncontrolled diabetes mellitus; mixed dyslipidemia can occur due to hepatitis B virus- or C virus-related nephrotic syndrome; and hypercholesterolemia can occur due to primary biliary cirrhosis or other cholestatic liver diseases. Consequently, the aforementioned liver diseases are potential causes of pseudohypona-
Hyponatremia is commonly observed using indirect ion-selective electrodes for serum electrolyte measurement. Therefore, the use of direct ion-selective electrodes or an osmometer for serum osmolality measurements is suggested to determine the true sNa concentration.

**Step 2. Evaluate the severity of clinical hyponatremia symptoms**

Symptomatic moderate or severe/profound hyponatremia should be treated promptly with hypertonic saline to improve symptoms, and this should be prioritized over further diagnostic testing. If acute management has been initiated or there is asymptomatic or mild hyponatremia, step 3 should be followed.

**Step 3. Measure urinary osmolality**

When urinary osmolality falls to 100 mOsm/kg or below, which indicates maximally diluted urine, excessive water and hypotonic food or fluid (e.g., beer, rice wine, liquid diet) should be discontinued.

**Step 4. Classify hyponatremia based on urine sodium concentration and extracellular fluid status**

The ECF status is divided into hypovolemia, euvolemia, or hypervolemia using the patient's history and physical examinations. A urine sodium level ≤30 mmol/L indicates low effective arterial volume, including hypovolemia and hypervolemia. For contracted ECF volume (hypovolemia), non-renal sodium loss including diarrhea, vomiting, or remote diuretics should be considered, while for expanded ECF volume (hypervolemia), liver cirrhosis, HF, and nephrotic syndrome should be considered. When the urine sodium level is >30 mmol/L, diuretics or kidney disease should be ruled out and ECF status should be assessed. For contracted ECF volume (hypovolemia), diuretics or renal sodium loss including renal or cerebral salt wasting should be considered, while for normal ECF volume (euvolemia), SIAD, secondary adrenal insufficiency, or hypothyroidism should be ruled out. The clinical assessment to determine the volume status is difficult as both the sensitivity (0.5–0.8) and specificity (0.3–0.5) are low. Therefore, additional modalities such as fractional excretion of sodium (with a cutoff of 1) or urea (with a cutoff of 35) or point-of-care ultrasound (POCUS) can be helpful for assessing volume status.
can provide additional assistance in patients receiving diuretics.\textsuperscript{57}

Step 5. Conduct a rapid adrenocorticotropic hormone test, assess thyroid function, and measure serum creatinine.

Coexisting factors, including adrenal insufficiency, severe hypothyroidism, and kidney failure, should be evaluated.

**CLINICAL SIGNIFICANCE OF HYponATREMIA IN LIVER DISEASE**

Hyponatremia has been linked to higher morbidity and mortality in patients with acute and chronic liver disease, acute-on-chronic liver failure (ACLF), or those awaiting LT.\textsuperscript{58,59}

**Liver cirrhosis**

Several studies have found that hyponatremia in liver cirrhosis is associated with a high incidence of liver-related complications, including a high prevalence of RA, HE, spontaneous bacterial peritonitis, and HRS; an increased need for massive paracenteses; and a shorter interval between paracenteses.\textsuperscript{3}

**Hepatic encephalopathy and hyponatremia**

Patients with acute hyponatremia have a higher incidence of neurological symptoms (such as headache, disorientation, confusion, focal neurological deficits, seizures, and in some cases, death due to brain herniation) than patients with chronic hyponatremia.\textsuperscript{60} Hypoosmolar hyponatremia expands astrocytes by moving water from the extracellular space into astrocytes to maintain osmotic balance; however, the expansion of brain cells is constricted due to the limited anatomical space.\textsuperscript{60,61} Accordingly, intracellular solutes are discharged in the opposite direction to reduce intracellular osmotic pressure. Intracellular potassium is excreted first, followed by organic osmotic pressure, including myoinositol, glutamine, choline, and taurine shifts.\textsuperscript{60,63} This protective adaptation mechanism can be observed in chronic hyponatremia and liver disease. The secondary increase in the intracellular glutamine content due to ammonia metabolism acts synergistically, mainly resulting in astrocyte edema and dysfunction of the glial-neuronal communication pathway.\textsuperscript{15,62,63}

Hyponatremia then further exacerbates astrocyte swelling and causes HE in patients with liver cirrhosis.\textsuperscript{43} Hyponatremia is a major risk factor for HE in patients with liver cirrhosis, increasing HE risk by 8.36 times.\textsuperscript{7} Therefore, monitoring sNa levels in patients with cirrhosis is critical for preventing or managing HE.

**Refractory ascites, acute kidney injury, and hepatorenal syndrome**

The pathogenesis of ascites is similar to that of hyponatremia due to excess water in the body, arterial vasodilation, arterial underfilling, and activation of the renin-angiotensin-aldosterone system and ADH.\textsuperscript{64-66} In addition, a large amount of body fluid may accumulate due to increased intestinal capillary permeability resulting from the decrease in albumin synthesis caused by decreased liver function.\textsuperscript{66} As much as 10% of patients with decompensated liver cirrhosis develop RA, characterized by severe ascites and requiring repeated large-volume paracenteses, even after receiving adequate diuretics and a salt-restricted diet.\textsuperscript{64-67} One study found the incidence of RA to be higher in patients with hyponatremia than in patients with normal concentrations of sNa (sNa level <130 mmol/L: 29.4% vs. sNa level >135 mmol/L: 13.5%, \(p<0.001\)). \textsuperscript{3} A study of American veterans showed that the 1-year mortality rate for patients with RA was close to 70%.\textsuperscript{58} Similarly, a study in Spain showed that the 1-year survival rate among patients with RA was only 31.6% and also showed that hyponatremia was an independent predictor of survival in patients with cirrhosis and RA.\textsuperscript{69}

Approximately 14–50% of patients with ascites and hyponatremia develop renal dysfunction in the form of acute renal injury or HRS.\textsuperscript{44,69,70} Renal failure is more common in patients who have hyponatremia with ascites, and hyponatremia is known to be an independent risk factor for acute renal injury (28% of those with sNa levels >135 mmol/L, 40% of those with sNa levels <130 mmol/L).\textsuperscript{3}

**Acute-on-chronic liver failure**

ACLF is defined as acute and rapidly progressive liver failure in patients with previously diagnosed or undiagnosed chronic liver disease, associated with multi-organ failure and very high short-term mortality.\textsuperscript{59,71} Approximately 25% of patients with ACLF have hyponatremia,\textsuperscript{39} and the frequency of hyponatremia is higher on the first day of hospitalization in pa-
Liver transplantation

A prospective cohort study showed that patients referred for consideration of LT with hyponatremia have a higher risk of early death regardless of cirrhosis severity, as assessed using the MELD score. Therefore, some researchers have advocated for rapid LT in patients with cirrhosis and a MELD score <21, persistent ascites, and hyponatremia. Another prospective multicenter study showed that incorporating the sNa level into the MELD score more accurately predicted survival compared to the MELD alone. As a result of these studies, the United Network for Organ Sharing incorporated the sNa level into the MELD score (MELD-Na) for organ allocation in January 2016.

The effect of pre-transplant hyponatremia on survival after transplantation is controversial. The largest study (n=19,537) conducted during the MELD era showed no significant reduction in survival after LT for patients with hyponatremia. In another study conducted in the US, no significant difference in the survival rate at 90 days after LT between recipients with normal sNa concentrations and those with hyponatremia was found. Another UK study revealed that the 3-year risk-adjusted mortality rate was higher in patients with pre-transplant hyponatremia than those with normal sNa levels. A small single-center study also showed a relative decrease in 3-month survival in patients with an sNa concentration <130 mmol/L compared to patients without hyponatremia. In addition, patients with hyponatremia had a high incidence of neurologic complications, renal failure, prolonged hospitalization in an intensive care unit, and increased ventilator requirements after LT. The impact of pre-transplant hyponatremia on posttransplant prognosis is still controversial; however, hyponatremia correction before transplantation is one of the methods used to reduce the risk of various complications.

Osmotic demyelination syndrome

Osmotic demyelination syndrome (ODS) is a rare but irreversible neurologic complication from excessive hyponatremia therapy that can result in death. When sNa levels return to normal, electrolytes and osmolality are restored in brain cells. Electrolytes are rapidly corrected, whereas organic osmolality is corrected slowly. This results in the shrinkage of glial cells, axonal shear damage, tight junction destruction, and cell death. ODS occurrence is difficult to predict; however, it has been established that individuals who are alcoholics, malnourished, or have advanced liver disease and are liver transplant recipients are at high risk for developing ODS, especially during overcorrection of chronic hyponatremia. Furthermore, the conditions that increase susceptibility to ODS, including malnutrition, hypokalemia, and hypoxia, are frequently observed in patients with liver disease. ODS symptoms can range from mild behavioral changes to dysarthria, dysphagia, ataxia, Parkinson’s disease, and widespread neurological deficits. According to the 2013 American guidelines, patients with advanced liver disease are classified as high risk for developing ODS; thus, a lower target of 4–6 mmol/L/day, with a maximum of 8 mmol/L in any 24-hour period, is recommended. Therefore, assessing the underlying disease and major risk factors for ODS is critical for the proper management of hyponatremia in patients with liver disease.

RECOMMENDATION OF MANAGEMENT

The approach to hyponatremia treatment in liver disease is similar to that used in the general population; however, some specific considerations are required in advanced liver disease. Hyponatremia management is guided by the severity of symptoms (asymptomatic or symptomatic [mild, moderate, severe]) and duration of hyponatremia (acute [<48 h] or chronic [≥48 h or unknown]). Hyponatremia treatment aims to prevent a further decrease in the sNa concentration, reduce intracranial pressure in patients at risk of brain prolapse, relieve hyponatremia symptoms, and prevent overcorrection. Hyponatremia management strategies in patients with liver disease are presented in Figure 3.

Symptomatic acute or chronic hyponatremia

The 2013 American guidelines and 2014 European guidelines recommend that patients with moderately or severely
symptomatic hyponatremia undergo treatment with hypertonic saline. In cases of severe symptomatic hyponatremia, rapid intermittent bolus regimens of hypertonic saline should be immediately infused to raise sNa levels by 4 to 6 mmol/L to improve cerebral edema. In cases of moderate symptomatic hyponatremia, rapid intermittent boluses or slow continuous infusions of hypertonic saline may be administered. The target correction rate is an sNa concentration of 5–9 mmol/L within the first 24 hours and 10–17 mmol/L or 130 mmol/L within 48 hours. However, for patients with decompensated liver cirrhosis, administration of hypertonic saline should be limited to severely symptomatic patients with life-threatening conditions such as cardio-respiratory distress, seizures, and coma because it may worsen volume overload or increases ascites and edema. Hypertonic saline is also used to increase sNa levels in patients with hyponatre-
hyponatremia before LT to prevent rapid hyponatremia correction after LT.\textsuperscript{83} The correction rate for hyponatremia should not exceed 8 mmol/L in any 24-h period in advanced liver disease because of the high risk for ODS.\textsuperscript{83}

### Asymptomatic acute hyponatremia

An absence of symptoms indicates that significant brain swelling has not yet occurred. Therefore, instead of administering hypertonic saline infusions, the etiology of hyponatremia must be assessed and cause-specific treatments be initiated. However, if the acute drop in sNa concentration exceeds 10 mmol/L, hypertonic saline should be administered to prevent a further decrease in the concentration.\textsuperscript{1} Hypertonic saline administration may be delayed if hyponatremia is corrected by water diuresis. Urine volume, low urine specific gravity, and low urine osmolality can help clinicians determine the hypertonic saline infusion volume. The treatment goal is to rapidly increase the sNa concentration to 4–6 mmol/L to relieve symptoms and prevent brain hernias.\textsuperscript{1}

### Asymptomatic chronic hyponatremia

Asymptomatic chronic hyponatremia does not require urgent correction; however, identifying and treating the underlying cause is critical. In liver cirrhosis, hyponatremia is mostly the chronic asymptomatic type due to brain adaptation. Therefore, it is essential to differentiate the cause of hyponatremia based on the ECF volume status.

Hypovolemic hyponatremia is caused by excessive use of diuretics and reduced effective volume due to gastrointestinal bleeding. Thus, hypovolemic hyponatremia must be corrected by discontinuing the diuretics and expanding the plasma volume using crystalloid fluids, albumin, etc.

Hypervolemic or dilutive hyponatremia is common and worsens as liver disease advances. Considering the etiology of hypervolemic hyponatremia, the ideal solution is to increase the excretion of water without solutes. Various management methods have been introduced and are discussed below. The treatment evidence is summarized in Table 2.

### Water restriction

Fluid restriction has been suggested as a first-line treatment for hyponatremia. For effective fluid restriction, water intake should be about 500 mL/day less than the sum of

### Table 2. Characteristics of the studied for the treatment of hyponatremia in liver disease according to use of tolvaptan or albumin infusion

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Design</th>
<th>Sample Size (n)</th>
<th>Definition of hyponatremia</th>
<th>Dose</th>
<th>Control group</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolvaptan</td>
<td>RCT</td>
<td>448</td>
<td>sNa &lt;135 mmol/L</td>
<td>Tolvaptan 15, 30, 60 mg/d</td>
<td>Placebo</td>
<td>See Figure SB</td>
</tr>
<tr>
<td>Cárdenas et al.\textsuperscript{90} (2012)</td>
<td>RCT</td>
<td>120</td>
<td>sNa &lt;135 mmol/L</td>
<td>Tolvaptan 15, 30, 60 mg/d</td>
<td>Placebo</td>
<td>See Figure SB</td>
</tr>
<tr>
<td>Rai et al.\textsuperscript{91} (2017)</td>
<td>RCT</td>
<td>50</td>
<td>sNa ≤130 mmol/L</td>
<td>Tolvaptan 15, 30, 60 mg/d</td>
<td>Methylprednisolone</td>
<td>See Figure SB</td>
</tr>
<tr>
<td>Wang et al.\textsuperscript{92} (2018)</td>
<td>RCT</td>
<td>98</td>
<td>sNa &lt;133 mmol/L</td>
<td>Tolvaptan 7.5, 15 mg/d</td>
<td>Placebo</td>
<td>See Figure SB</td>
</tr>
<tr>
<td>Tang et al.\textsuperscript{93} (2020)</td>
<td>RCT</td>
<td>143</td>
<td>sNa &lt;135 mmol/L</td>
<td>Tolvaptan 7.5, 15 mg/d</td>
<td>Placebo</td>
<td>See Figure SB</td>
</tr>
</tbody>
</table>

Albumin infusion

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Design</th>
<th>Sample Size (n)</th>
<th>Definition of hyponatremia</th>
<th>Dose</th>
<th>Control group</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bajaj et al.\textsuperscript{107} (2018)</td>
<td>Cohort</td>
<td>1,121</td>
<td>sNa ≤130 mmol/L</td>
<td>125 g (IQR 100, 400 g)</td>
<td>NA</td>
<td>See Figure SD</td>
</tr>
<tr>
<td>Bai et al.\textsuperscript{117} (2022)</td>
<td>Cohort</td>
<td>394</td>
<td>sNa &lt;135 mmol/L</td>
<td>40 g (IQR 10, 380 g)</td>
<td>NA</td>
<td>See Figure SD</td>
</tr>
<tr>
<td>Zaccherini et al.\textsuperscript{109} (2023)</td>
<td>RCT</td>
<td>149</td>
<td>sNa &lt;135 mmol/L</td>
<td>20% HA in 50-mL vials: 40 g twice a week for 2 weeks and then, 40 g weekly</td>
<td>NA</td>
<td>See Figure SD</td>
</tr>
</tbody>
</table>

RCT, randomized control trial; sNa, serum sodium; SMT, standard medical therapy; IQR, interquartile range; HA, human albumin.
urine output and insensible loss (1–1.5 L/day). Fluid restriction should be considered if there are neurological symptoms or if the sNa concentration is <120 mmol/L; however, the role of fluid restriction is limited in patients with mild, asymptomatic hyponatremia. The water restriction method is associated with poor patient compliance, making it difficult to achieve sNa normalization. A good indicator of adequate fluid restriction is a change in the sNa concentration within the first 24–48 hours. However, if sNa levels do not increase within the first 48–72 hours, other options should be considered.

**Correction of hypokalemia**

Hypokalemia may result from increased urine loss due to diuretic treatment or increased gastrointestinal fluid loss in patients with diarrhea or vomiting. Hypokalemia correction is recommended for two reasons: hypokalemia increases renal ammonia synthesis, and concomitant alkalosis increases the fraction of bound ammonia in plasma, which can lead to HE. When osmotically active potassium is corrected, replenished potassium enters the cells, and intracellular sodium moves in the opposite direction, increasing the sNa concentration and osmotic pressure without external sodium administration. Hence, since potassium is as osmotically active as sodium, excessive sNa and hypokalemia corrections can cause severe complications such as ODS due to overcorrection.

**Vasopressin receptor antagonists or vaptans**

Key question: In hyponatremic patients with liver disease, does the vaptan group show more effective sodium correction than the no vaptan group?

Vasopressin receptor antagonists or vaptans are non-peptide drugs that inhibit ADH action on the V1a, V1b, and V2 receptors. The V2 receptor responsible for water absorption is located in the basolateral membrane of the renal collecting duct, and a blockade of this receptor can cause water diuresis and increase the sNa concentration (Fig. 4). The V2 selective blocker of ADH effectively improves the sNa concentration in SIAD or HF cases with elevated vasopressin.

Tolvaptan is the only selective V2 receptor antagonist that can be administered orally, and several studies have shown that tolvaptan significantly increases the sNa concentration and improves ascites in patients with liver cirrhosis, but does not improve the survival rate. In a multicenter clinical trial that followed patients with HF, SIAD, and liver cirrhosis longitudinally for an average of 1.9 years, the sNa concentration significantly increased in patients with HF, SIAD, and liver cirrhosis after taking tolvaptan; however, the increase in the sNa concentration in patients with liver cirrhosis was slower than that in patients with HF and SIAD. To evaluate the effect of tolvaptan in terms of sodium correction, we performed a database search of Ovid MEDLINE and conducted a meta-analysis of existing randomized controlled trials (Fig. 5A). Tolvaptan was found to be effective at correcting hyponatremia in patients with liver disease (Fig. 5B and Table 2). Additionally, the beneficial effect of vaptan on ascites was confirmed in three meta-analyses. A recent cohort study showed that an increase in sNa levels after one month of tolvaptan treatment could positively affect the risk of death in patients with cirrhosis and hyponatremia. However, a previous meta-analyzes reported no change in the short-term and long-term survival of patients with cirrhosis after treatment with vaptan. For patients with cirrhosis and hyponatremia at baseline, survival rates tended to improve after treatment with vaptan, though it was not statistically significant. The most common side effects of tolvaptan include thirst, polyuria, and dry mouth. Serious side effects such as dehydration with hypotension, dehydration with dizziness, syncope, and acute renal failure can also occur. In an open-label phase 4 study aimed at evaluating the efficacy

![Figure 4. Mechanism of action of vaptan. ADH, antidiuretic hormone; AQP2, aquaporin-2; AQP3, aquaporin-3; AQP4, aquaporin-4.](https://doi.org/10.3350/cmh.2023.0090)
and safety of tolvaptan in autosomal dominant polycystic kidney disease, aspartate transaminase and alanine transaminase levels were elevated in two patients (1.7%), and these abnormal findings improved after maintaining the dose or temporarily discontinuing tolvaptan. Therefore, the US Food & Drug Administration has limited tolvaptan use to less than 30 days and recommends that it not be used in patients with underlying cirrhosis due to the risk of liver failure and death. However, it should be noted that the dose of tolvaptan in this study was 4–8 times the dose commonly used to treat hyponatremia. The European Association for the Study of the Liver clinical practice guidelines for decompen-
sated cirrhosis recommends the routine use of tolvaptan for correction of low sodium levels only in controlled clinical trials.\textsuperscript{101} Korea’s Ministry of Food and Drug Safety has approved tolvaptan for hyponatremic patients with HF or SIAD, but not for patients with liver disease.\textsuperscript{102} The Japanese Society of Gastroenterology does not mention tolvaptan use for correcting hyponatremia; however, tolvaptan has been approved as an additional drug for fluid retention in patients with liver cirrhosis and is recommended as an additional drug for patients with ascites that is refractory to treatment with existing diuretics (furosemide and spironolactone).\textsuperscript{103} The use of vaptans in patients with reduced liver function is restricted in most countries, and caution is advised when administering vaptans because relevant studies on long-term survival are lacking.

**Albumin**

Key question: In hyponatremic patients with liver disease, does the albumin group show more effective sodium correction than the no albumin group?

---

**Figure 5.** Continued.
Albumin replacement is another treatment option that can improve hyponatremia associated with liver cirrhosis. Furthermore, albumin replacement in patients with cirrhosis has been widely used to manage various complications such as spontaneous bacterial peritonitis, HRS, ascites, and HE.\(^{104-109}\) Previous authors have argued that intravenous albumin infusions can increase the sNa concentration through expanding the intravascular volume, thus increasing free water clearance in the urine.\(^{102,110}\) Regarding the pathogenesis of hyponatremia in liver cirrhosis, the sNa concentration can be increased by maintaining colloidal osmotic pressure and affecting the inflammatory pathway, improving the hypervolemic circulation state, and removing inflammatory factors.\(^{111}\)

Most previous studies have evaluated the role that albumin replacement plays in preventing hyponatremia after large-volume paracentesis in cirrhosis hyponatremia, but relatively few studies have evaluated the role that albumin plays in hyponatremia treatment.\(^{112-117}\) In a retrospective multicenter study of 1,125 patients with liver cirrhosis accompanied by hyponatremia (regardless of large-volume paracentesis) at the time of admission, the delta values of the sNa concentrations between the albumin replacement group and the non-albumin replacement group were compared, showing significantly higher delta values in the albumin replacement group.\(^{113}\) In addition, the post-hoc analysis of the ANSWER study, which compared the group receiving long-term albumin treatment (20% human albumin in a 50 mL vial: 40 g twice a week for 2 weeks, then 40 g weekly thereafter) to the group receiving standard medical treatment, showed at the 3-month follow-up that albumin was associated with sNa normalization over time.\(^{115}\) The most recent meta-analysis evaluating the efficacy of albumin replacement also suggested that albumin replacement can be helpful in the prevention and treatment of hyponatremia in liver cirrhosis.\(^{116}\) However, recommendations on the use of albumin replacement therapy in the management of cirrhosis with hyponatremia vary according to clinical guideline. While some suggest that albumin should be used to prevent or treat hyponatremia in liver cirrhosis,\(^{103}\) other reports recommend that albumin infusions be carefully evaluated due to lack of data.\(^{113,118}\)

We searched the Ovid MEDLINE database for studies evaluating the efficacy of albumin infusions for sodium correction in patients with liver disease. Only one RCT and two cohort studies were found that evaluated the use of albumin in the treatment of hyponatremia for patients with liver cirrhosis. The results of this one RCT are replicated here, and we performed a meta-analysis of the two cohort studies (Fig. 5C and Table 2).\(^{113,115,117}\) Our analysis (Fig. 5D) showed that the use of albumin was significant for treating hyponatremia that had already occurred. In most studies, the use of albumin resulted in significant improvements in hyponatremia; however, long-term outcomes, including the survival rate, showed a heterogeneous relationship. In a cohort study conducted by Bajaj et al.\(^{113}\) (2018) that evaluated 1,126 patients with hyponatremia, the albumin group showed resolution of hyponatremia and a higher 30-day survival rate. However, a post hoc analysis of the ATTIRE trial conducted in 2021, which included 206 hyponatremic patients, showed that albumin infusions were not associated with mortality.\(^{114}\) In another cohort study conducted by Bai et al.\(^{117}\) (2022), which included 339 patients with liver cirrhosis, albumin infusions were found to effectively improve serum sodium levels during hospitalization in patients with cirrhosis and hyponatremia, but did not have a significant effect on long-term mortality.

Although high-quality studies on hyponatremia treatment are lacking, it appears that albumin injections may help prevent and treat hyponatremia in patients with cirrhosis. However, these findings must be validated through a well-designed, large-scale study, and the optimal dosages and durations of treatment and their effect on outcomes, including survival rates, must be further evaluated.

**Perioperative management of patients awaiting liver transplantation**

Pre-transplant hyponatremia is associated with prolonged hospitalization, prolonged intensive care unit admission, and neurologic complications.\(^{76-78,119,120}\) Efforts to optimize sNa levels to >125 mmol/L before LT are needed, as patients with severe hyponatremia before transplantation are more prone to neurological complications such as ODS after transplantation.\(^{76,121,122}\) The risk of ODS after LT in patients with liver disease increases with lower baseline sNa levels.\(^{76}\) Patients undergoing LT are at increased risk for rapid sNa correction due to fluid shifts that occur during surgery from the intraoperative administration of intravenous crystalloids, blood products, and sodium bicarbonate.\(^{10,83,123}\) However, despite the importance of correcting hyponatremia, no definitive protocol for perioperative management of hyponatremia and no
correction threshold for sNa levels are currently available.\textsuperscript{123}

The incidence of ODS, a severe complication caused by rapid sNa correction after LT, ranges from 0.5% to 29%.\textsuperscript{7,6,124} Symptoms such as quadriplegia (up to 45%) and seizures (27–36%) occur within 1–2 weeks after LT.\textsuperscript{125,126} Since ODS is associated with poor prognosis,\textsuperscript{76,125,126} various methods have been used to prevent rapid correction of sNa. LT recipients receive a large amount of sodium-containing intravenous fluids, such as packed red blood cells, platelet concentrates, and fresh frozen plasma; thus rapid sNa correction may occur after surgery.\textsuperscript{10,83,123} To minimize this rapid correction, 0.45% saline or 5% glucose water can be used instead of 0.9% normal saline.\textsuperscript{127} Additionally, prothrombin complex concentrates with low sNa levels can be used instead of fresh frozen plasma.\textsuperscript{127} Finally, for patients who have received or will receive high intraoperative sodium loads through blood transfusions and intravenous fluids, continuous veno-venous hemofiltration using dilute solutions before or during surgery.

\textbf{Figure 6.} Management of a patient with hyponatremia and end-stage liver disease (ESLD) for liver transplantation (LT) (Figure modified from Praharaj and Anand. J Clin Exp Hepatol 2022;12:575-594).\textsuperscript{129}
may be considered for managing rapid changes in sNa levels, however, relevant data are very limited.

Since hyponatremia improves when fundamental liver function is restored after LT, the occurrence of severe complications such as ODS due to rapid changes in sNa must be monitored. Some studies have shown that mortality due to ODS after LT is 40% at 3 months and 63% at 1 year, and up to 84% of surviving patients have been reported to suffer from permanent sequelae. Desmopressin administration as a rescue measure is useful when unintended overcorrection of hyponatremia occurs. One proposed approach for the perioperative management of hyponatremia in patients awaiting LT is summarized in Figure 6.

CONCLUSION

Hyponatremia is a pervasive electrolyte disorder in advanced chronic liver disease. Fundamental treatment strategies include water restriction, hypokalemia correction, and diuretic discontinuation. Hypertonic saline can be considered in acutely symptomatic hyponatremia; however, caution is advised given the potential for fatal complications such as ODS and worsening ascites. Furthermore, although vasopressin receptor antagonists and albumin replacement can be used to correct hyponatremia in liver cirrhosis, supportive studies are lacking, and individual countries may have controversies regarding their use. Although LT is ideal for managing hyponatremia in patients with advanced chronic liver disease, multidisciplinary approaches to perioperative management are essential for correcting hyponatremia.

Authors’ contribution

JYR and SHB contributed to manuscript research and writing. SHB and SK contributed to the conceptualization and critical review and editing of the manuscript.

Acknowledgements

This study was supported by a grant from the National Research Foundation of Korea (no. 2021R1C1C1008966).

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES


Received : Jun. 21, 2023 / Revised : Jul. 31, 2023 / Accepted : Aug. 8, 2023

Editor: Bo Hyun Kim, National Cancer Center, Korea

Review

Carbon ion radiotherapy in the treatment of hepatocellular carcinoma

Hwa Kyung Byun¹, Changhwan Kim², and Jinsil Seong²

¹Department of Radiation Oncology, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin, ²Department of Radiation Oncology, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea

Hepatocellular carcinoma (HCC) is a highly lethal cancer with limited treatment options and poor prognosis. Carbon ion radiotherapy (CIRT) has emerged as a promising treatment modality for HCC due to its unique physical and biological properties. CIRT uses carbon ions to target and destroy cancer cells with a high precision and efficacy. The Bragg Peak phenomenon allows precise dose delivery to the tumor while minimizing damage to healthy tissues. In addition, the high relative biological effectiveness of carbon ions can be shown against radioresistant and hypoxic tumor areas. CIRT also offers a shorter treatment schedule than conventional radiotherapy, which increases patient convenience and compliance. The clinical outcomes of CIRT for HCC have shown excellent local control rates with minimal side effects. Considering its physical and biological properties, CIRT may be a viable option for complex clinical scenarios such as patients with poor liver function, large tumors, re-irradiation cases, and tumors close to critical organs. Further research and larger studies are needed to establish definitive indications for CIRT and to compare its efficacy with that of other treatment modalities. Nevertheless, CIRT offers a potential breakthrough in HCC management, providing hope for improved therapeutic outcomes and reduced treatment-related toxicities. (Clin Mol Hepatol 2023;29:945-957)

Keywords: Carbon ion radiotherapy; Hepatocellular carcinoma; Radiotherapy; Particle therapy

INTRODUCTION

Liver cancer ranks as the eighth and fifth leading cause of cancer-related mortality among females and males, respectively, in the United States.¹ In Korea, it stands as the seventh most prevalent cancer type. With a 5-year survival rate of 38.7%, liver cancer is regarded as one of the fatal types of cancer. Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer.² A multidisciplinary approach has been employed in the management of HCC, with treatment options ranging from surgical resection and liver transplantation to minimally invasive ablation techniques, transarterial therapies, radiation therapy, and systemic therapy.³⁵ The choice of treatment depends on factors, such as disease stage, liver function, and overall patient health, with the ultimate goal of achieving optimal therapeutic outcomes and improving the patient’s quality of life. Due to its high recurrence and poor prognosis rates, the development of effective treatment strategies for HCC has become a pressing. In recent years, carbon ion radiotherapy (CIRT) has emerged as a promising treatment option for patients with HCC, offering potential advantages over traditional modalities such as sur-
gery, chemotherapy, and conventional radiotherapy.

CIRT is a type of particle radiotherapy that utilizes carbon ion beams to precisely target and destroy cancer cells. The key advantages of CIRT are its unique physical and biological properties. Carbon ions deposit more energy within a small volume of tissue, causing more damage to cancer cells and having a better biological effect than photons or protons. Furthermore, carbon ions possess a characteristic dose distribution that enables the delivery of a high dose of radiation to the tumor while minimizing damage to the surrounding healthy tissues. The application of CIRT in the treatment of HCC is based on its ability to overcome some of the challenges posed by conventional therapies.

This article provides an in-depth review on the use of CIRT as a treatment option for HCC. It covers the underlying physical and biological principles of CIRT, clinical outcomes of patients with HCC treated with CIRT, and a comparison of CIRT with other available treatment options. This article also suggested the clinical scenarios where CIRT could yield benefits in the management of HCC.

### PHYSICAL ADVANTAGES OF CIRT IN THE TREATMENT OF HCC

The most notable physical property of the CIRT is the Bragg Peak. This special physical characteristic distinguishes particle therapy from X-ray therapy. As an X-ray beam traverses matter, its intensity gradually decreases owing to the absorption or scattering of photons from the beam. In contrast, when a particle beam passes through matter, it deposits most of its energy in the final millimeters of its trajectory while slowing down. This results in a steep increase in the absorbed dose, known as the Bragg peak (Fig. 1). This characteristic enables precise dose delivery to the target, while minimizing radiation exposure to healthy tissues located before and beyond the target site.

The Spread-Out Bragg Peak (SOBP) concept is employed to treat tumors with larger volumes. Peak energy deposition, the Bragg Peak, occurs at a specific depth within the tissue and can be calculated based on the initial ion energy. The SOBP is generated by superimposing multiple Bragg Peaks of varying energies and intensities, effectively creating a broader and uniform dose distribution within the tumor. SOBP en-
sures that the entire tumor volume receives a consistent therapeutic dose while preserving the advantageous properties of the Bragg Peak, such as dose conformity and reduced damage to the surrounding healthy tissue. Representative radiation treatment plans for HCC using carbon ion and X-ray beams are shown in Figure 2. Carbon ion beams can produce a more precise and conformal dose distribution to the tumor than X-ray beams while minimizing the exposure of the surrounding normal liver tissue to radiation. In Shiba et al.’s retrospective study, a comparison was made between the dosimetric outcomes of patients with HCC who underwent intensity-modulated radiotherapy using X-ray beams and those who received CIRT, both administered at a dose of 60 Gy. The study showed that patients treated with CIRT had significantly lower mean liver doses and a lower percentage of normal liver volume exposed to radiation doses exceeding 5 Gy, 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy than those who underwent intensity-modulated radiotherapy. Patients with HCC often present with compromised liver function, which makes the protection of healthy liver tissue a crucial aspect of HCC treatment. Therefore, this feature makes CIRT a favorable option for HCC management.

**BIOLOGICAL ADVANTAGES OF CIRT IN THE TREATMENT OF HCC**

**Strong biological effectiveness**

Linear energy transfer (LET) refers to the rate of energy loss experienced by particle beams as they penetrate tissues. Photons, electrons, and protons beams are low-LET radiations that exhibit sparse ionization. Carbon ions, alpha particles and fast neutrons beams are high-LET radiations that exhibit dense ionization. Heavy ions, such as carbon ions, have a high atomic mass and possess a high LET. The LET and relative biological effectiveness (RBE) of radiation are closely associated. High-LET radiation tends to have a higher RBE than low-LET radiations. The RBE is defined as the ratio of the doses required by two different types of radiation to cause the same level of effect. The RBE of photons is set at 1. In proton therapy, a consistent RBE value of 1.1 is widely accepted. In contrast, the RBE of carbon ions is not a constant value, but rather depends on their position within the treatment beam. As the carbon ions penetrate further into the target lesion, their RBE increases. These features offer therapeutic benefits because the biological effects of carbon ion beams intensify as they progress deeper into the tumor area. The local RBE values for carbon ions can reach as high as 2.0–3.5. Moreover, at the entry site (normal tissue), the RBE value is lower than that of the target lesions. This disparity in RBE between cancerous and normal tissue expands the therapeutic window, as it enhances the biological effects within the target region while minimizing damage to normal tissue.

**Strong effect on hypoxic tumor**

Tumor hypoxia has been recognized as a key mechanism that causes radioresistance in cancer cells. Chronic hypoxia results from excessive proliferation of cancer cells accompa-
nied by poor vasculature. The increased distance between cells and their nearest blood vessels restricts oxygen diffusion from the tumor microvessels to the surrounding tissue. Low-LET radiation predominantly induces DNA damage by generating free radicals; this is known as the indirect action of radiation. The generation of free radicals is promoted by the presence of oxygen, whereas high-LET radiation directly strikes the DNA molecule, disrupting its molecular structure; this is called the direct action of radiation. This extensive damage caused by high-LET radiation is less dependent on the oxygen concentration. Consequently, carbon ion beams demonstrate superior efficacy in hypoxic tumors. Given the common occurrence of hypoxia in the intratumor regions of HCC, caused by abnormal microvasculature and uncontrolled cell proliferation leading to low oxygen levels, CIRT can offer valuable benefits for managing HCC. Hypoxia in HCC is known to be associated with tumor aggressiveness, chemoresistance, and immunotherapy resistance, making CIRT’s superior effectiveness in hypoxic conditions relevant for HCC treatment.  

Short treatment schedule

Fractionated irradiation is an important concept in conventional radiotherapy that uses X-rays. Several biological effects contribute to the advantages of fractionated radiation. Between irradiations, damaged normal tissues recover as sublethal damage is repaired (repair) and the cells repopulate (repopulation). Additionally, the time between irradiation sessions allows tumor cells to progress into the radiosensitive phases of the cell cycle (redistribution) and allows the surviving hypoxic tumor cells to become oxygenated (reoxygenation). The “4Rs”—repair, repopulation, redistribution, and reoxygenation—form the fundamental rationale for radiation fractionation.

However, the 4Rs have diminished significance for high-LET beams, including CIRT. For example, sublethal damage repair and repopulation of the tissue is suppressed in CIRT. Carbon ion beams are less affected by the cell cycle or cellular oxygenation than X-rays. The implications of the 4Rs, and thereby the effect of fractionated irradiation, on CIRT are minor compared to those of conventional X-ray therapy. Furthermore, due to the sharper physical dose distribution of CIRT, critical organs are exposed to reduced radiation doses, allowing for CIRT hypo-fractionation strategies. The application of hypofractionated radiotherapy in HCC has benefits in several aspects. In X-ray therapy, the reduction of fractionation, known as stereotactic body radiotherapy, has been shown to not only have clinical anti-tumor effects but also minimize the impact of radiation-induced lymphopenia in HCC.  

Furthermore, hypofractionated radiotherapy leads to a significant activation of the immune system, thereby enhancing the efficacy of immune checkpoint inhibitors in HCC treatment. To implement hypofractionated radiotherapy, ensuring safety is crucial, and CIRT’s physical and biological characteristics offer the necessary assurance in this regard. Historically, CIRT has implemented fewer fractions than conventional X-ray therapy. In general, conventional radiotherapy using X-ray or proton beam therapy uses a larger number of scheduled fractions, ranging from 10 to 35 in published reports. In contrast, current CIRT protocols mostly use 2 or 4 fractions for tumors located at a distance from the gastrointestinal tracts, while 12 fractions or more were typically used for tumors in close proximity to the gastrointestinal tracts (Table 1). In a multi-institutional retrospective study conducted by the Japan Carbon Ion Radiation Oncology Study Group, short course CIRT with 2 or 4 fraction regimens has demonstrated a curative local effect while maintaining acceptable treatment-related toxicities for HCC.

In summary, the physical properties of CIRT, specifically, the superior dose distribution resulting from the Bragg Peak, enable a high dose concentration in the tumor area while minimizing radiation exposure to normal organs. Furthermore, biological properties such as high RBE within the target area and low RBE in non-target areas expand the therapeutic window of carbon-ion beams in comparison to proton and photon beams. The efficacy of CIRT in addressing tumor hypoxia helps overcome radioresistance and aid in controlling large hypoxic tumors. Additionally, a shorter treatment schedule due to the fewer fractions enhances patient convenience and increases compliance in patients with comorbidities or those who must travel long distances for treatment.

CLINICAL OUTCOMES OF CIRT FOR HCC

Treatment outcomes of CIRT for HCC

The efficacy and feasibility of CIRT for HCC have been investigated in several prospective phase I and II studies (Table
<table>
<thead>
<tr>
<th>Author, yr, center</th>
<th>No. of pts</th>
<th>Total dose, Gy (RBE)/fractions</th>
<th>Median follow-up, months (range)</th>
<th>Tumor size</th>
<th>No. of tumors</th>
<th>Macrovacular invasion</th>
<th>Liver function (Child-Pugh class)</th>
<th>Local control</th>
<th>Progression-free survival</th>
<th>Overall survival</th>
<th>Grade 3+ Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective phase 1/2 trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kato et al., 2004, NIRS</td>
<td>24</td>
<td>49.5–79.5 Gy/15 fr</td>
<td>Median 5 cm</td>
<td>One: 21 pts Two: 3 pts</td>
<td>NR</td>
<td>A: 16 pts B: 8 pts</td>
<td>1 yr: 92% 3 yr: 81% 5 yr: 81%</td>
<td>NR</td>
<td>1 yr: 92% 3 yr: 50% 5 yr: 2.5%</td>
<td>Acute: gr 3 skin toxicity, 1 pt; gr 3 leukocytopenia and thrombocytopenia, 5 pts Late: gr 3 thrombocytopenia, 3 pts (needed of variceal bleed and hepatic failure)</td>
<td></td>
</tr>
<tr>
<td>Kasuya et al., 2017, NIRS</td>
<td>126</td>
<td>69.6 Gy/12 fr, 58 Gy/8 fr, 52.8 Gy/4 fr</td>
<td>Median 4 cm</td>
<td>Single: 103 tumors Multiple: 30 tumors</td>
<td>Present in 23 or 133 tumors</td>
<td>A: 97 pts B: 29 pts</td>
<td>1 yr: 94.7% 3 yr: 91.4% 5 yr: 90.0%</td>
<td>NR</td>
<td>1 yr: 90.3% 3 yr: 50.0% 5 yr: 25.0%</td>
<td>Acute: gr 3 skin toxicity, 3 pts Late: gr 3 skin, 3 pts; gr 3 pleural effusion, 1 pt</td>
<td></td>
</tr>
<tr>
<td>Shibuya et al., 2019, Gunma</td>
<td>21</td>
<td>60 Gy/4 fr</td>
<td>Median 4.8 cm</td>
<td>Single: 20 pts Multiple: 1 pt</td>
<td>None</td>
<td>A: 21 pts</td>
<td>1 yr: 100% 2 yr: 92.3% 2 yr: 81.0% 2 yr: 100%</td>
<td>NR</td>
<td>1 yr: 90.5% 2 yr: 80.0%</td>
<td>Acute: none Late: gr 3 cholecystitis and encephalopathy, 2 pts; gr 3 other toxicity (not specified), 1 pt</td>
<td></td>
</tr>
<tr>
<td>Shibuya et al., 2021, Gunma</td>
<td>35</td>
<td>52.8 Gy/4 fr, 60 Gy/4 fr</td>
<td>Median 3.5 cm</td>
<td>Single: 34 pts Multiple: 1 pt</td>
<td>None</td>
<td>A: 29 pts B: 6 pts</td>
<td>2 yr: 92.6% 3 yr: 76.5% 4 yr: 76.5% 2 yr: 45.7% 3 yr: 33.8% 4 yr: 29.5%</td>
<td>2 yr: 82.8% 3 yr: 76.7% 4 yr: 69.4%</td>
<td>2 yr: 82.8% 3 yr: 76.7% 4 yr: 69.4%</td>
<td>Acute: none Late: gr 3 hepatobiliary toxicity, 2 pts</td>
<td></td>
</tr>
<tr>
<td>Hong et al., 2023, SPHIC</td>
<td>23</td>
<td>55–70 Gy/10 fr</td>
<td>Median 4.3 cm</td>
<td>Single: 15 pts Multiple: 8 pts</td>
<td>Present in 6 pts</td>
<td>A: 23 pts</td>
<td>1 yr: 100% 3 yr: 94.4% 5 yr: 94.4% 1 yr: 73.6% 3 yr: 59.2% 5 yr: 37.0%</td>
<td>1 yr: 91.3% 3 yr: 81.9% 5 yr: 67.1%</td>
<td>1 yr: 91.3% 3 yr: 81.9% 5 yr: 67.1%</td>
<td>Acute: gr 3 leukocytopenia, 2 pts Late: gr 3 stomach bleeding, 2 pts</td>
<td></td>
</tr>
<tr>
<td>Author, yr, center</td>
<td>No. of pts</td>
<td>Total dose, Gy (RBE)/fractions</td>
<td>Median follow-up, months (range)</td>
<td>Tumor size</td>
<td>No. of tumors</td>
<td>Macrovascular invasion</td>
<td>Liver function (Child-Pugh class)</td>
<td>Local control</td>
<td>Progression-free survival</td>
<td>Overall survival</td>
<td>Grade 3+ Toxicities</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>-------------------------------</td>
<td>---------------------------------</td>
<td>------------</td>
<td>---------------</td>
<td>----------------------</td>
<td>----------------------------------</td>
<td>--------------</td>
<td>-------------------------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Imada et al., 2010, NIRS&lt;sup&gt;5&lt;/sup&gt;</td>
<td>64</td>
<td>52.8 Gy/4 fr</td>
<td>Porta hepatis: 34 (6–90)</td>
<td>3.7 cm</td>
<td>Single: 15 pts</td>
<td>Porta hepatis group: present in 16 pts</td>
<td>Porta hepatis group: A: 16 pts</td>
<td>Porta hepatis group A: 3 yr</td>
<td>87.8%</td>
<td>3 yr: 44.4%</td>
<td>Acute: gr 3 liver toxicity, 3 pts, gr 3 hematologic toxicity, 6 pts (porta hepatis) gr 3 liver toxicity, 8 pts, gr 3 hematologic toxicity, 8 pts (non-porta hepatis) Late: NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-porta hepatis: 41 (11–98)</td>
<td>3.7 cm</td>
<td>Multiple: 3 pts</td>
<td>Non-porta hepatis group: present in 29 pts</td>
<td>Porta hepatis group: B: 2 pts</td>
<td>Porta hepatis group B: 3 yr</td>
<td>87.8%</td>
<td>3 yr: 22.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-porta hepatis group:</td>
<td>Porta hepatis group C: 3 yr</td>
<td>3 yr: 5.6%</td>
<td>5 yr: 23.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 yr: 34.8%</td>
<td>Non-porta hepatis group C: 5 yr</td>
<td>3 yr: 60.9%</td>
<td>5 yr: 34.8%</td>
<td></td>
</tr>
<tr>
<td>Komatsu et al., 2011, HIBMC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>101</td>
<td>52.8 Gy/4 fr, 52.8 Gy/8 fr, 66 Gy/10 fr, 76 Gy/20 fr</td>
<td>31</td>
<td>&lt;5 cm: 81 tumors 2–10 cm: 22 tumors &gt;10 cm: 5 tumors</td>
<td>Single: 81 pts</td>
<td>Present in 19 of 108 tumors</td>
<td>Porta hepatis group A: 78 pts</td>
<td>Porta hepatis group A: 5 yr</td>
<td>93%</td>
<td>5 yr: 36.3%</td>
<td>Acute: none Late: gr 3 elevation of transaminase level, 3 pts; gr 3 subcutaneous panniculitis, 1 pt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Multiple: 20 pts</td>
<td></td>
<td>Porta hepatis group B: 20 pts</td>
<td>Porta hepatis group B: 5 yr</td>
<td>93%</td>
<td>5 yr: 36.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Porta hepatis group C: 3 pts</td>
<td>Porta hepatis group C: 5 yr</td>
<td>93%</td>
<td>5 yr: 36.3%</td>
<td></td>
</tr>
<tr>
<td>Habermehl et al., 2013, HY&lt;sup&gt;9&lt;/sup&gt;</td>
<td>6</td>
<td>40 Gy/4 fr</td>
<td>11 (3.4–12.7)</td>
<td>Median 3.5 cm</td>
<td>One: 3 pts</td>
<td>Single: 31 pts</td>
<td>Present in 6 pts</td>
<td>A: 27 pts</td>
<td>2 yr: 89.2%</td>
<td>2 yr: 51.3%</td>
<td>Acute: none Late: gr 3 encephalopathy, 3 pts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Two: 2 pts</td>
<td>Multiple: 1 pt</td>
<td></td>
<td>B: 4 pts</td>
<td>2 yr: 82.3%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Author, yr, center</th>
<th>No. of pts</th>
<th>Total dose, Gy (RBE/fractions)</th>
<th>Median follow-up, months (range)</th>
<th>Tumor size</th>
<th>No. of tumors</th>
<th>Macrovascular invasion</th>
<th>Liver function (Child-Pugh class)</th>
<th>Local control</th>
<th>Progression-free survival</th>
<th>Overall survival</th>
<th>Grade 3+ Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shibuya et al., 2018, J-CROS</td>
<td>174</td>
<td>48 Gy/2 fr, 52.8/4 fr, 60/4 fr</td>
<td>20.3 (2.9–103.5)</td>
<td>Median 3.0 cm</td>
<td>One: 157 pts Two: 15 pts Three: 2 pts</td>
<td>None</td>
<td>A: 153 pts B: 20 pts</td>
<td>1 yr: 94.6% 3 yr: 81.0%</td>
<td>NR</td>
<td>1 yr: 95.4% 3 yr: 73.3%</td>
<td>Acute: gr 3 dermatitis, 2 pts; gr 3 elevation of AST, 1 pt Late: gr 3 dermatitis, 4 pts; gr 3 myopathy, 1 pt; gr 3 rib fracture, 1 pt; gr 4 dermatitis, 1 pt</td>
</tr>
<tr>
<td>Shibuya et al., 2018, Gunma</td>
<td>68</td>
<td>52.8 Gy/4 fr, 60.0 Gy/4 fr</td>
<td>33.5 (3.9–83.1)</td>
<td>Sarcoopenia: 3 cm Non-sarcoopenia: 3.6 cm</td>
<td>NR</td>
<td>NR</td>
<td>A: 17 pts B: 5 pts Non-sarcoopenia: A: 40 pts B: 6 pts</td>
<td>3 yr: Sarcoopenia: 81% Non-sarcoopenia: 72%</td>
<td>3 yr: Sarcoopenia: 46% Non-sarcoopenia: 30%</td>
<td>3 yr: Sarcoopenia: 66% Non-sarcoopenia: 77%</td>
<td>Acute: none Late: gr 3 encephalopathy, 2 pts</td>
</tr>
<tr>
<td>Shibuya et al., 2019, Gunma</td>
<td>31</td>
<td>52.8 Gy/4 fr, 60.0 Gy/4 fr, 60.0 Gy/12 fr</td>
<td>43 (4–84)</td>
<td>Median 3.4 cm</td>
<td>Single: 31 pts</td>
<td>None</td>
<td>A: 29 pts B: 2 pts</td>
<td>3 yr: 80% 3 yr: 51% 3 yr: 88%</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shibuya et al., 2020, Gunma</td>
<td>11</td>
<td>52.8 Gy/4 fr, 60.0 Gy/4 fr, 60.0 Gy/12 fr</td>
<td>36.4 (4.3–86.2)</td>
<td>Median 5.3 cm</td>
<td>NR</td>
<td>Present in 11 pts</td>
<td>A: 10 pts B: 1 pt</td>
<td>3 yr: 78%</td>
<td>3 yr: 18%</td>
<td>3 yr: 64%</td>
<td>Acute: none Late: gr 3 bone fracture, 1 pt</td>
</tr>
<tr>
<td>Yasuda et al., 2019, NIRS</td>
<td>57</td>
<td>45 Gy/2 fr</td>
<td>54 (7–103)</td>
<td>Median 3.3 cm</td>
<td>Single: 56 pts Multiple: 1 pt</td>
<td>None</td>
<td>A: 51 pts B: 6 pts</td>
<td>1 yr: 98% 3 yr: 91% 5 yr: 91%</td>
<td>NR</td>
<td>1 yr: 97% 3 yr: 67% 5 yr: 43%</td>
<td>Acute: gr 3 skin toxicity, 2 pts Late: none</td>
</tr>
<tr>
<td>Fujita et al., 2022, NIRS</td>
<td>69</td>
<td>45 Gy/2 fr, 48 Gy/2 fr, 52.8 Gy/4 fr</td>
<td>51.6 (3.1–130.0)</td>
<td>Median 2.7 cm</td>
<td>Single: 66 pts Multiple: 3 pts</td>
<td>NR</td>
<td>A: 68 pts B: 1 pt</td>
<td>2 yr: 92.1% 5 yr: 89.7%</td>
<td>2 yr: 77.7% 5 yr: 50.0%</td>
<td>2 yr: 83.7% 5 yr: 55.7%</td>
<td>Acute: none Late: none</td>
</tr>
<tr>
<td>Hiroshima et al., 2023, NIRS</td>
<td>58</td>
<td>45 Gy/2 fr, 48 Gy/2 fr, 52.8 Gy/4 fr, 60 Gy/4 fr</td>
<td>20.5 (2.7–108)</td>
<td>Median 3.2 cm</td>
<td>Single: 52 tumors Multiple: 16 tumors</td>
<td>Present in 8 of 69 tumors</td>
<td>B: 58 pts</td>
<td>1 yr: 96.4% 2 yr: 96.4%</td>
<td>1 yr: 38.6% 2 yr: 6.9%</td>
<td>1 yr: 80.4% 2 yr: 46.0%</td>
<td>Acute: gr 3 hepatotoxicity Late: none</td>
</tr>
</tbody>
</table>
The first prospective phase I trial was reported from the National Institute of Radiological Sciences in 2004 exploring dose escalation from 49.5 Gy (RBE) in 15 fractions to 79.5 Gy (RBE) in 15 fractions. No severe adverse effects or treatment-related deaths were reported. The local control rate was 81% at both 3 and 5 years. The National Institute of Radiological Sciences reported the combined results of phase I and II trials in 2017. The maximum tolerated doses were determined at 69.6, 58.0, and 52.8 Gy (RBE) in 12, 8, and 4 fractions, respectively, and 52.8 Gy (RBE) in 4 fractions was established as the recommended dose regimen for the two phase II studies. Gunma University has reported the results of prospective trials conducted in 2019 and 2022. Regimens of 52.8 Gy (RBE) and 60 Gy (RBE) in four fractions were used and showed 92.3% local control rate at 2 years and 76.5% local control rate at 4 years. Late grade 3 hepatobiliary toxicity occurred in 2 patients with no grade 4 or more toxicity. The Shanghai Proton and Heavy Ion Center has reported the outcomes of a phase I trial. Dose levels ranged from 55 to 70 Gy (RBE) in 10 fractions, and no dose-limiting toxicity was observed. The 5-year local control rate was 94.4%.

Several retrospective studies regarding CIRT for HCC have been also conducted (Table 1). A multi-institutional retrospective study conducted by the Japan Carbon Ion Radiation Oncology Study Group showed the results of 174 patients with HCC treated with CIRT with regimens of 48 Gy (RBE) in two fractions, 52.8 Gy (RBE) in 4 fractions, or 60 Gy (RBE) in 4 fractions. The 3-year local control rate was 81.0% and the 3-year overall survival rate was 73.3%. Acute grade 3 toxicities included dermatitis in two patients and elevation of AST in one patient. Late grade 3 toxicities included dermatitis in four patients, myopathy in one patient, and rib fractures in one patient. Grade 4 late dermatitis occurred in one patient. Upon reviewing the retrospective studies outlined in Table 1, the local control rate at 5 years was generally close to or more than 90% overall. Acute or late toxicities of grade 3 or higher were observed in a few cases. The recent series uses 2 or 4 fractions for tumors distant from gastrointestinal tracts and 12 or more fractions for tumors close to them, optimizing dose delivery and minimizing adverse effects.

CIRT in comparison with other modalities

Most existing literature focuses solely on CIRT. A few small retrospective studies have examined the effectiveness of
CIRT compared with other treatment modalities. Further studies with larger numbers of patients or prospective designs are needed to directly compare CIRT with other modalities.

Shiba et al. compared CIRT with transarterial chemoembolization for the treatment of single HCC using propensity score matching. After analyzing 17 matched pairs, the 3-year overall survival, local control, and progression-free survival rates in the CIRT vs. transarterial chemoembolization groups were 88% vs. 58% (P<0.05), 80% vs. 26% (P<0.01), and 51% vs. 15% (P<0.05), respectively. These results revealed that CIRT resulted in more favorable clinical outcomes than transarterial chemoembolization, although larger patient numbers are required to confirm the results.

Fujita et al. compared CIRT with radiofrequency ablation as initial treatments for early-stage HCC. Among the 560 patients examined, 69 underwent CIRT and 491 underwent radiofrequency ablation. After propensity score matching, the CIRT group had significantly lower cumulative intrasubsegmental recurrence rates than the radiofrequency ablation (2-year, 12.6% vs. 31.7%; 5-year, 15.5% vs. 49.6%, P=0.004). However, local recurrence rates, progression-free survival, and overall survival were comparable between the two groups. Notably, no adverse events grade 3 or higher were observed in the CIRT group, while 1.2% of patients showed grade 3 adverse events in the radiofrequency ablation group.

**Suggested special scenarios for the application of CIRT in HCC**

Although definitive indications for CIRT in HCC have yet to be established, its superior dose profiles make CIRT a viable choice in complex clinical cases that are unsuitable for traditional X-ray therapy. In particular, CIRT can reduce radiation-related hepatotoxicity while maintaining effective tumor control. Radiation-induced liver disease (RILD) is a form of subacute liver injury triggered by radiation and is one of the most dreaded complications of radiotherapy for liver cancer. To minimize the risk of RILD, stereotactic body radiation therapy using X-rays for HCC <4 cm, 4–10 cm, and >10 cm were 96.15%, 90.90%, and 76.47%, respectively. However, local recurrence rates, progression-free survival, and overall survival were comparable between the two groups. Notably, no adverse events grade 3 or higher were observed in the CIRT group, while 1.2% of patients showed grade 3 adverse events in the radiofrequency ablation group.

CIRT offers a promising alternative treatment for patients with poor liver function, who are often ineligible for radiotherapy or other local therapies. Hiroshima et al. demonstrated this in their study of 58 patients with HCC with Child-Pugh B liver function. Only one patient experienced grade 3 acute hepatotoxicity with no acute or late grade 4 or higher adverse events following CIRT administered at doses of 45 Gy (RBE) or 48 Gy (RBE)/2 fractions, as well as 52.8 Gy (RBE) or 60 Gy (RBE) in 4 fractions.

CIRT also offers advantages for the treatment of patients with large tumors. In general, as the tumor size increases, the radiation target volume also increases, as does the radiation exposure of the normal liver. In addition, large tumors often show a poorer response to radiation than small tumors. A retrospective study revealed that the response rates to stereotactic body radiation therapy using X-rays for HCC <4 cm, 4–10 cm, and >10 cm were 96.15%, 90.90%, and 76.47%, respectively. Given its superior dose distribution and high RBE, CIRT may significantly improve the treatment outcomes in large HCCs, while minimizing the risk of RILD.

In cases requiring re-irradiation, the cumulative radiation dose to the liver increases when considering both the previous radiotherapy and the re-irradiation doses. As the cumulative mean radiation dose to the liver increases, the risk of hepatotoxicity also increases. CIRT can reduce the radiation dose to the normal liver during re-irradiation by providing excellent radiation dose distribution. Tomizawa et al. reported no instances of grade 4 or higher toxicity among 41 patients who underwent repeat CIRT for intrahepatic HCC recurrence. The prescribed dose was 52.8 Gy or 60.0 Gy (RBE) in 4 to 12 fractions. The change in the albumin-bilirubin score before and after the second CIRT was also insignificant, suggesting minimal liver function deterioration after re-irradiation using CIRT.

Another beneficial scenario for CIRT is when HCC is in close proximity to a critical organ. For instance, HCC in the caudate lobe typically has a poor prognosis owing to its proximity to the portal trunk and inferior vena cava, which facilitates early systemic spread. Additionally, its deep location in the liver...
and proximity to major vessels pose technical challenges for surgical resection, radiofrequency ablation, and ethanol injection in the caudate lobe.\(^\text{39}\) Furthermore, the complex arterial blood supply of the caudate lobe makes transarterial chemoembolization difficult to achieve local tumor control.\(^\text{40}\) In such cases, radiotherapy can be an effective local treatment option for HCC in the caudate lobe, being less influenced by the anatomical features of the caudate lobe. Various radiotherapy techniques, such as intensity-modulated radiotherapy, stereotactic body radiotherapy, and particle beam therapy, have been attempted for patients with difficult-to-treat HCC with other local therapy modalities.\(^\text{41}\) CIRT has also been applied in situations where HCC is located in the caudate lobe. Okazaki et al.\(^\text{19}\) reported the results of CIRT in the treatment of HCC located in the caudate lobe. The study demonstrated no local recurrence, and only two instances of grade 2 or 3 late adverse events were observed among the nine patients. Furthermore, for HCCs adjacent to the porta hepatitis, anatomical resection can be invasive because of its large resection volume. CIRT has demonstrated excellent outcomes when applied in situations where the HCC is close to the porta hepatitis. Imada et al.\(^\text{19}\) compared the CIRT results between patients with HCC located within 2 cm of the main portal vein and those with HCC far from the porta hepatitis. Their findings revealed no significant differences in overall survival, local control, or toxicity between the two groups, highlighting the effectiveness and safety of CIRT in the porta hepatitis group just as in the non-porta hepatitis group. Notably, biliary stricture associated with CIRT was not observed.

**LIMITATIONS OF CIRT IN HCC**

One primary limitation is the scarcity of clinical evidence comparing the CIRT to other treatment modalities.\(^\text{42}\) As CIRT is a relatively new and specialized approach, there are limited large-scale clinical trials for HCC. This lack of data hinders a comprehensive assessment of its long-term effectiveness and safety in treating diseases.

Another challenge arises when HCC is located near the gastrointestinal tract. The highly conformal radiation field produced by carbon-ion beams is affected by various uncertainties, including bowel motion and bowel gas. There is a possibility that focal high dose can affect the gastrointestinal mucosa, potentially leading to complications such as ulceration, bleeding, and perforation.\(^\text{43}\) Although studies have shown low occurrences of gastrointestinal complications with CIRT, there remains a potential risk due to the impact of high-intensity doses.

Moreover, CIRT’s physical and biological properties present further limitations. Carbon ion beams have a more rapid lateral fall-off around the target volume compared to proton beams, resulting in smaller lateral penumbra. However, beyond the distal end of the peak, carbon ion beams exhibit a fragmentation tail caused by a small dose deposited due to nuclear interactions and particle fragmentation, whereas proton beams show almost no dose deposition.\(^\text{45}\) Since the tail contains only fragments with a low atomic number, the biological effect of this fragmentation tail and its clinical implications are minimal.

Range uncertainty in the beam path length is another major concern. The stopping position of the carbon beam is sensitive to density variations along the beam path. Due to the steep dose gradient, anatomical changes, including organ movements or the changes in bowel gas, can significantly impact the robustness of the treatment plan. To mitigate these uncertainties, robust treatment planning and motion management techniques have been developed.\(^\text{46}\)

Furthermore, RBE of CIRT possesses uncertainty. RBE is affected by numerous factors, including measured endpoint, dose, dose rate, dose per fractionation, number of fractions, particle charge and velocity, oxygen concentration, and cell-cycle phase. While biophysical models such as the local effect model (LEM) or microdosimetric kinetic model (MKM) are used to determine the RBE of CIRT, theoretical modeling of the biological effects of heavy ions remains a challenging task due to the complexity and limited knowledge of the physical, chemical, and biological processes involved.\(^\text{47}\) Despite CIRT’s successful application for several decades in the real world, research on these biophysical models continues to be an active area of investigation.\(^\text{48}\)

**CONCLUSION**

The traditional treatment approaches for HCC often exhibit limited efficacy and substantial side effects. CIRT is an advantageous solution owing to its unique physical and biological properties. The Bragg Peak, a key attribute of CIRT, enables precise delivery of high-dose radiation to the tumor site.
while minimizing exposure to the surrounding healthy tissue. Furthermore, CIRT’s high LET contributes to an elevated RBE. This capability enhances the destruction of cancer cells, particularly in hypoxic tumors that tend to resist traditional radiotherapy. Several prospective and retrospective studies have demonstrated the benefits of CIRT for HCC management. Compared with conventional therapies, CIRT exhibits excellent local control and reduces adverse effects. Its effectiveness in treating larger tumors along with its suitability for patients with compromised liver function, those requiring re-irradiation, or those with tumors located near the clinical organs, further highlights CIRT’s potential as a groundbreaking therapeutic strategy. However, despite the promising results from prospective I/II and small retrospective studies, it is essential to acknowledge the current lack of phase III clinical trials directly comparing CIRT with other treatment modalities in HCC. The superiority of CIRT in effectiveness and safety over conventional therapies has not yet been definitively demonstrated. Well-designed phase III clinical trials are warranted in the future to provide robust evidence and establish CIRT as a leading therapeutic option for HCC. Through these endeavors, we can establish more definitive guidelines for the implementation of CIRT in HCC treatment, paving the way for improved patient outcomes.

Authors’ Contribution
Conception, design of the study, and literature review and analysis: HK Byun and J Seong; drafting and critical revision and editing, and final approval of the final version: HK Byun, C Kim and J Seong.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


2017;96:e9249.


Current evidence and the potential role of proton beam therapy for hepatocellular carcinoma

Sung Uk Lee and Tae Hyun Kim

1Center for Proton Therapy, National Cancer Center, Goyang; 2Center for Liver and Pancreatobiliary Cancer, National Cancer Center, Goyang, Korea

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death, and external beam radiation therapy has emerged as a promising approach for managing HCC. Proton beam therapy (PBT) offers dosimetric advantages over X-ray therapy, with superior physical properties known as the Bragg peak. PBT holds promise for reducing hepatotoxicity and allowing safe dose-escalation to the tumor. It has been tried in various clinical conditions and has shown promising local tumor control and survival outcomes. A recent phase III trial demonstrated the non-inferiority of PBT in local tumor control compared to current standard radiofrequency ablation in early-stage HCC. PBT also tended to show more favorable outcomes compared to transarterial chemoembolization in the intermediate stage, and has proven effective in-field disease control and safe toxicity profiles in advanced HCC. In this review, we discuss the rationale, clinical studies, optimal indication, and future directions of PBT in HCC treatment. (Clin Mol Hepatol 2023;29:958-968)

Keywords: Carcinoma, hepatocellular; Radiotherapy; Proton therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy arising in the liver and is increasing in incidence with significant impacts on morbidity and mortality. While surgical management remains the primary treatment option, the majority of patients are not appropriate candidates due to advanced disease at diagnosis or poor expected postoperative liver function or surgical morbidity. This renders alternative local treatments critical for the long-term management of these patients in a variety of settings. Non-surgical treatment options include percutaneous ablation, transarterial chemoembolization (TACE), selective internal radiotherapy (SIRT), and external beam radiation therapy (EBRT). EBRT modalities include 3D conformal radiotherapy (3D-CRT), intensity-modulated radiation therapy (IMRT), volumetric-modulated arc therapy (VMAT), stereotactic body radiation therapy (SBRT), proton beam therapy (PBT), and carbon ion radiotherapy (CIRT). Because key risk factors for the development of HCC include cirrhosis of any cause and hepatitis B or C virus infections, EBRT has had a very limited role in the treatment of HCC in patients whose livers are mostly cirrhotic or poorly functioning, as these patients are most vulnerable to radiation-induced liver disease (RILD). With recent advances in EBRT technology, photon EBRT such as IMRT demonstrated clinical efficacy without increasing RILD in the management of HCC, and the role of PBT has emerged as a powerful technique with its superior physical property in ab-
Relative dose delivery to tumors while sparing the uninvolved liver and other nearby critical organs. Here, we review the current evidences and potential roles of PBT according to the Barcelona Clinic Liver Cancer (BCLC) staging classifications in the management of HCC.

RATIONALE AND DOSIMETRIC BENEFIT OF PBT

The liver is one of the important radiation dose-limiting organs during EBRT, with RILD risk associated with the irradiated liver volume. Thus, minimizing the radiation dose to the remaining normal liver during EBRT for HCC is crucial. Conceptually, more sophisticated EBRT techniques, including IMRT, VMAT, and PBT, may improve tumor control in HCC patients by delivering a high radiation dose to the tumor while minimizing the dose to the remaining normal liver, thereby minimizing impairment of the remaining hepatic reserve.

Regarding the physical characteristic, the X-ray dose delivered decreases gradually along the beam path with increasing beam depth. Thus, an exit dose is inevitably delivered to adjacent normal tissues, and even IMRT or VMAT cannot avoid low-dose delivery at the distal area of the beam path. In contrast, a proton beam has a finite range of energy deposition and loses most of its energy within a very short distance at the end of the beam range. This results in a sharp rise and fall in energy absorption, known as the Bragg peak. Therefore, PBT has been considered to have superior physical properties compared to other X-ray-based EBRT techniques, delivering a high radiation dose to the tumor while minimizing the radiation dose delivered to the remaining normal liver, thereby minimizing impairment of the remaining hepatic reserve. Figure 1 presents the radiation dose distribution of various treatment plans using X-ray and PBT for representative 5.7 cm sized HCC case in segment 8. PBT showed the advantage of less radiation exposure in the remaining normal liver, especially in the low-dose area. Regarding the effect of PBT according to tumor size, previous dosimetric analyses have demonstrated that the larger the tumor size, the greater the benefit of PBT in decreasing the risk of RILD.

Several dosimetric comparison studies have demonstrated the dose volumetric benefits of PBT compared to 3D-CRT and/or IMRT for HCC. Li et al. found that PBT reduced the mean liver dose (D_{mean}), the fractional volume of the liver receiving doses greater or equal to 10 Gy (V_{10}), 20 Gy (V_{20}), 30 Gy (V_{30}) and better spared non-liver organ-at-risks (OAR) (stomach and kidney) than 3D-CRT or IMRT. Wang et al. demonstrated similar results, with significant reductions in the V_{30} of the remaining normal liver and the D_{mean} as well as reduced stomach, duodenum, heart, and spinal cord by PBT compared to IMRT. Kim et al. compared dose-volume histogram data among helical-IMRT (H-IMRT), VMAT, and PBT for HCC and found that PBT provided equal planning target volume (PTV) tumor coverage, conformity index, and homogeneity index values and significantly better sparing of the liver (D_{mean} and V_{10}) to V_{30} for remaining normal liver and non-liver OARs (D2 cm³ of the stomach and spinal cord) compared to H-IMRT and VMAT. Even though the difference between PBT and either H-IMRT or VMAT in the irradiated volumes of the remaining normal liver at higher doses (from V_{30} to V_{50}) may not have been clinically significant (less than 3%), PBT significantly reduced the irradiated liver volume at dose levels below V_{35} (about 50% of the prescribed dose). These data suggest that PBT may be superior to other EBRT modalities, including 3D-CRT, H-IMRT, and VMAT, in reducing the risk of RILD, and it may also have an advantage during dose escalation.

PROTON BEAM THERAPY TECHNIQUES

Numerous published data demonstrating the efficacy of PBT for HCC have utilized passive scattering (PS)-PBT treatment techniques. Pencil beam scanning (PBS)-PBT may provide more conformal dose distribution and superior normal tissue sparing by the intensity modulation of PBT, enabling improved dose optimization and conformity along the proximal edge compared to PS-PBT. PBS-PBT is considered more sensitive to organ motion with respiration than passive scattering due to the interplay effect. Recently, published retrospective series demonstrated the feasibility, safety, and

Abbreviations:
HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; SIRT, selective internal radiotherapy; EBRT, external beam radiation therapy; IMRT, intensity modulated radiation therapy; VMAT, volumetric-modulated arc therapy; SBRT, stereotactic body radiation therapy; PBT, proton beam therapy; CIRT, carbon ion therapy; OAR, organ at risk; RILD, radiation-induced liver disease; SOBP, spread-out Bragg peak.

efficacy of PBS-PBT for treating HCC. Although only small retrospective series have been reported, most studies achieved high local tumor control (around 95% at one year) and low toxicity profiles.\textsuperscript{16,17} Yoo et al.\textsuperscript{18} compared the outcomes of HCC patients treated with either PS-PBT or PBS-PBT. After propensity score matching, they revealed no difference in toxicity, tumor control, or survival between patients treated with PS-PBT or PBS-PBT.\textsuperscript{18} As a majority of newer PBT centers have PBS-PBT capability, an increase in data supporting PBS-PBT for HCC treatment is anticipated.

**RE-IRRADIATION**

In selected cases, PBT can be the optimal EBRT technique for delivering a second course of radiation with a curative dose to a previously irradiated liver by preserving the remaining healthy liver and the adjacent critical abdominal organs.\textsuperscript{19} Early reports of Hashimoto et al.\textsuperscript{20} published in 2006 demonstrated the safety and feasibility of proton re-irradiation for recurrent HCC patients (n=27), and treatment-related toxicities of grade 3 or higher were observed in about 18% (n=5), of which 2 developed acute hepatic failure and the remaining 3 had late injuries (1 rib fracture and 1 bile duct stenosis). McDuff et al.\textsuperscript{21} (n=49) reported the more recent experiences

---

**Figure 1.** Radiation dose distributions of treatment plans for hepatocellular carcinoma using X-ray 3D-conformal RT (A). X-ray intensity modulated RT-volumetric modulated arc therapy (B). Proton beam therapy-passive scattering (C). Proton beam therapy-pencil beam canning (D) and the Dose-volume histogram graph of each technique (E). PBS, pencil beam scanning; IMRT, intensity modulated radiation therapy; VMAT, volumetric-modulated arc therapy.
confirming the safety and efficacy of proton re-irradiation for liver malignancies with the median follow-up of 10.5 months since re-irradiation. With regard to toxicity, only 2 patients (4.1%) experienced grade 3 toxicity of radiation-induced liver disease after reirradiation. Oshiro et al. retrospectively assessed 83 patients who received liver re-irradiation with protons, including 15 patients treated with three or more definitive EBRT courses. For most patients, repetitive PBT is the only possible treatment option because of the lack of other local therapeutic options. Patients were treated with similar PBT doses of 70 gray-equivalent (GyE) at re-irradiation, following an initial course of 71 GyE. The most commonly used dose schemes were 72.6 GyE in 22 fractions (33%), 66 GyE in 10 fractions (33%), and 74 Gy in 27 fractions (19%) (mean dose per fraction 3.3 GyE). Second-course planning considerations generally included maintaining a normal liver volume of more than 500 mL. This second course of PBT with definitive doses resulted in a median OS of 61 months from the time of the first treatment course, with no severe radiation-induced liver dysfunction or other acute toxicities. 

COMPARISON WITH OTHER EBRT TECHNIQUES

While PBT has been established as an acceptable local therapeutic option for early-to-advanced HCC, there still is a lack of high-quality evidence to guide recommendations for when proton therapy should be clearly preferred over photon therapy. Cheng et al. compared 110 HCC patients treated with either photon EBRT (n=55) or PBT (n=55) with curative intent after propensity matching. About half of the patients had vascular invasion. Cox regression analysis revealed a significant survival benefit (P=0.032, hazard ratio [HR]=0.56, 95% confidence interval [CI]: 0.33–0.96) and lower risk of RILD (11.8% vs. 36%, P=0.004) in the PBT group compared to the photon group. Qi et al. performed a meta-analysis to compare charged particle therapy versus photon therapy for HCC patients. The pooled OS was significantly higher for charged particle therapy than for conventional photon EBRT with improvements in progression-free survival and local control, while comparable efficacy was found between charged particle therapy and SBRT in OS, progression-free survival and local control. High-grade acute and late toxicity associated with charged particle therapy was lower than that of conventional photon EBRT and SBRT. Hasan et al.

compared PBT (n=71) with SBRT (n=918) in stage I-II HCC patients in the National Cancer Database. The results showed that PBT was independently associated with longer survival than SBRT (HR=0.48, 95% CI: 0.29–0.78), despite being delivered to HCC patients with multiple poor prognostic factors. NRG GI003 (NCT03186898) is an ongoing randomized trial to compare PBT versus conventional photon EBRT for HCC.

CIRT, a type of particle beam therapy, is known to have potential advantages with higher radiobiological effectiveness (RBE), offering therapeutic benefits in hypoxic or radioresistant tumor cells. Thus, CIRT has broadened its clinical application and was recently attempted in HCC. Despite the potential benefits of CIRT with high RBE values, the clinical outcome of CIRT appears to be similar to that of PBT, with about 80–90% local tumor control and 50% OS at three years.

From a treatment planning perspective, dosimetric studies suggest that PBT is advantageous for normal liver-sparing in patients with tumors >3 cm in specific locations or for larger tumors (>5 cm) in patients with good baseline liver function.

This expert panel recommended that proton therapy be strongly considered in the following scenarios: i) normal liver dose constraints cannot be met with photon therapy; ii) Child-Pugh B or greater cirrhosis based on data showing that low doses to the normal liver are associated with hepatotoxicity and unfavorable outcomes with PBT; iii) larger tumor size; iv) smaller uninvolved liver volume (e.g., <800 cm³), common in more severely cirrhotic patients and after partial liver resection; v) high tumor-to-liver ratio; vi) multiple number of tumors; and vii) prior radiation therapy to the liver.

CLINICAL BENEFIT OF PBT FOR HEPATOCELLULAR CARCINOMA

Early-to-intermediate-stage HCC (BCLC stage 0-B)

Early-to-intermediate-stage HCC encompasses the largest subgroup of patients with HCC. The recommended early-stage treatments are surgical resection, ablation, and trans-

plantation, and the expected five-year overall survival (OS) rate ranges from 50 to 70%.\textsuperscript{4,34-36} The standard therapeutic approach for patients with intermediate-stage disease is TACE, and the expected median survival time is about 30 months.\textsuperscript{4,37} PBT demonstrated an excellent local control rate reaching 85–95% and a comparable OS rate of more than 50% at 3–5 years after PBT in patients who received the current standard treatment for early-to-intermediate-stage HCC (Table 1).\textsuperscript{27,38-50}

PBT may have several advantages, such as non-invasiveness, use in locations unsuitable for other therapy, and non-echogenicity. Tumor compared to other local treatments, such as surgical resection and percutaneous ablative therapy, including radiofrequency ablation (RFA). The first phase 3 randomized controlled trial compared PBT and RFA in small recurrent/residual HCC (size <3 cm, number ≤2) and demonstrated that the local control effect of PBT was not inferior to that of RFA (PBT 86.5% vs. RFA 78.3% at 4-year, \( P=0.114 \)) in HCC patients.\textsuperscript{49} Crossover was allowed if the assigned treatment was technically infeasible, and PBT showed better feasibility than RFA with significantly lower crossover rate (8.3% vs. 26.4%, \( P=0.004 \)). The most common treatment-related toxicity was radiation pneumonitis (32.5%) for PBT and increased alanine aminotransferase levels (96.4%) and abdominal pain (30.4%) for RFA, respectively. PBT was tolerable and safe, consistent with the known profile. The associated good feasibility and comparable clinical outcomes suggest that PBT may be a promising treatment option for small HCCs.

TACE is a common treatment option for intermediate-stage or multiple HCCs, but local tumor control is often disturbed by the complex arterial blood supply of the tumor, such as collaterals.\textsuperscript{51} In general, tumor control by PBT is not compromised by the complexity of the tumor blood supply. Bush et al.\textsuperscript{52} recently reported their final results of a prospective randomized clinical trial comparing PBT with TACE in unresectable HCC. PBT was associated with better PFS and LC compared to TACE and even associated with fewer posttreatment hospitalization days, and reduced cost of treatment.

PBT seems to have excellent long-term local tumor control in treatment-naive HCC patients, ranging from 87% to 94%, with an OS rate ranging from 66 to 69%, which is comparable to other recommended first-line treatments. Fukuda et al.\textsuperscript{53} reported 5-year outcomes in 129 treatment-naive HCC patients after delivering a total of 66.0–77.0 GyE of PBT in 10–35 fractions. The 5-year local tumor control and OS rates were 94% and 69% for patients with 0/A stage disease (n=9/21) and 87% and 66% for patients with B-stage disease (n=34), respectively. Kim et al.\textsuperscript{54} reported the clinical outcomes of 46 treatment-naive HCC patients treated with PBT and showed similar results, with a 5-year freedom from local progression rate of 92.7% and OS rate of 69.2%.

Since most PBT data from early-to-intermediate-stage HCC were obtained from patients with recurrent or residual HCC, the recently developed 2022 Korean Liver Cancer Association-National Cancer Center Korea practice guidelines for the management of HCC described the role of EBRT including PBT as an effective local treatment option limited to small recurrent HCC.\textsuperscript{55} However, PBT has demonstrated similar efficacy in terms of local control, survival, and toxicity in treatment-naive HCC patients compared to those with recurrent HCC, as the data mentioned above. Hence, we may have to assume that PBT has a potential role as a first-line therapy for treatment-naive HCC patients as well as those with recurrent or residual HCC. Overall, the data suggest that PBT could be an effective alternative or complementary local treatment for early-to-intermediate-stage HCC where other local treatments, such as surgical resection, ablative therapy, and TACE, might be unsuitable or ineffective, and could be a potential first-line treatment in treatment-naive HCC patients. In addition, PBT was an effective way to increase the chances of curing localized HCC in liver transplantation candidates as definitive or bridging therapy while they wait for transplantation with less morbidity compared to TACE.\textsuperscript{52}

**Advanced-stage HCC (BCLC stage C)**

The recommended treatment for advanced-stage HCC, BCLC C, is systemic therapy, such as sorafenib, with an expected median survival time of about 10 months. A recent randomized trial comparing atezolizumab plus bevacizumab to sorafenib showed that the combination of atezolizumab and bevacizumab significantly improved objective responses and OS compared to sorafenib.\textsuperscript{56} However, the objective response was still around 20–30%, and relatively selected patients, such as only those in the Child-Pugh A class, participated and more than 50% of the patients experienced serious adverse events (grade 3–4 toxicity).\textsuperscript{56}

Nevertheless, local treatments, such as PBT, still may have a role in advanced HCC, especially in HCC with vascular invasion. A randomized controlled study compared combination
<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Stage mUICC</th>
<th>Stage BCLC</th>
<th>Vascular invasion (+)</th>
<th>Dose/Fx</th>
<th>EQD2</th>
<th>FFLP (3 yr)</th>
<th>OS (3 yr)</th>
<th>IHF</th>
<th>Toxicity (≥Gr3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kawashima et al.(^{38}) (2005) (n=30)</td>
<td>Phase II</td>
<td>I (30%)</td>
<td>0-B (60%)</td>
<td>C (40%)</td>
<td>40%</td>
<td>76 GeY/20 Fx</td>
<td>87.4 Gy</td>
<td>96% (2 yr)</td>
<td>66% (2 yr)</td>
<td>60%</td>
</tr>
<tr>
<td>Chiba et al.(^{35}) (2005) (n=162)</td>
<td>Retrospective</td>
<td>I (41%)</td>
<td>I (43%)</td>
<td>III (16%)</td>
<td>6%</td>
<td>50–84 GeY/10–24 Fx</td>
<td>62.5–94.5 Gy</td>
<td>90%</td>
<td>45%</td>
<td>85% (acute) 3.1% (late) (≥Grade 2)</td>
</tr>
<tr>
<td>Mizumoto et al.(^{40}) (2008) (n=53)</td>
<td>Retrospective</td>
<td>I (32%)</td>
<td>II (30%)</td>
<td>III (38%)</td>
<td>28%</td>
<td>72.6 GeY/22 Fx</td>
<td>80.5 Gy</td>
<td>86%</td>
<td>45.1%</td>
<td>54.7% 0%</td>
</tr>
<tr>
<td>Fukumitsu et al.(^{41}) (2009) (n=51)</td>
<td>Retrospective</td>
<td>I (61%)</td>
<td>0-A (38%)</td>
<td>B (13%)</td>
<td>26%</td>
<td>60 GeY/10 Fx</td>
<td>91.3 Gy</td>
<td>94.5%</td>
<td>49.2%</td>
<td>56.9% 5.9% Rib Fx Z% RP</td>
</tr>
<tr>
<td>Komatsu et al. (2011) (n=205)(^{27})</td>
<td>Retrospective</td>
<td>I (4%)</td>
<td>II-IV (96%)</td>
<td>A (38%)</td>
<td>6%</td>
<td>72.6–77 GeY/22–31 Fx</td>
<td>78.3–80.5 Gy</td>
<td>88.1%</td>
<td>50%</td>
<td>44.7% 2.1% GIT</td>
</tr>
<tr>
<td>Nakayama et al.(^{42}) (2011) (n=47)</td>
<td>Retrospective</td>
<td>I (43%)</td>
<td>II (36%)</td>
<td>III (21%)</td>
<td>14%</td>
<td>72.6–77 GeY/22–31 Fx</td>
<td>78.3–80.5 Gy</td>
<td>88.1%</td>
<td>50%</td>
<td>44.7% 2.1% GIT</td>
</tr>
<tr>
<td>Kim et al.(^{43}) (2015) (n=27)</td>
<td>Phase I</td>
<td>II (30%)</td>
<td>A (48%)</td>
<td>B (37%)</td>
<td>60 Gy</td>
<td>65 Gy</td>
<td>71.4%</td>
<td>25%</td>
<td>74.1% 0%</td>
<td></td>
</tr>
<tr>
<td>Hong et al.(^{44}) (2016) (n=44)</td>
<td>Phase II</td>
<td>A/B (50%)</td>
<td>58 GeY/15 Fx</td>
<td>67.1 Gy</td>
<td>95% (2 yr)</td>
<td>63% (2 yr)</td>
<td>40% (2 yr)</td>
<td>2.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chadha et al.(^{45}) (2019) (n=46)</td>
<td>Retrospective</td>
<td>A-B</td>
<td>0%</td>
<td>67.5 GeY/15 Fx</td>
<td>97.7 Gy</td>
<td>77% (2 yr)</td>
<td>67% (2 yr)</td>
<td>13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al.(^{46}) (2017) (n=71)</td>
<td>Retrospective</td>
<td>I (21%)</td>
<td>A (69%)</td>
<td>B (31%)</td>
<td>66 GeY/10 Fx</td>
<td>91.9 Gy</td>
<td>89.9%</td>
<td>74.4%</td>
<td>69%</td>
<td>0%</td>
</tr>
<tr>
<td>Author</td>
<td>Study design</td>
<td>Stage mUICC</td>
<td>Stage BCLC</td>
<td>Vascular invasion (+)</td>
<td>Dose/Fx</td>
<td>EQD2</td>
<td>FFLP (3 yr)</td>
<td>OS (3 yr)</td>
<td>IHF</td>
<td>Toxicity (≥Gr3)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>------------</td>
<td>-----------------------</td>
<td>---------</td>
<td>------</td>
<td>-------------</td>
<td>-----------</td>
<td>-----</td>
<td>-----------------</td>
</tr>
<tr>
<td>Kim et al. (2019)</td>
<td>Retrospective</td>
<td>I (7%)</td>
<td>I (7%)</td>
<td>7%</td>
<td>66 GyE/10 Fx</td>
<td>91.9 Gy</td>
<td>92.4 (5 yr)</td>
<td>67.9% (5 yr)</td>
<td>71.4%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II (42%)</td>
<td>II (42%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III (44%)</td>
<td>III (44%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV (7%)</td>
<td>IV (7%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al. (2020)</td>
<td>Phase II</td>
<td>I (36%)</td>
<td>I (36%)</td>
<td>2%</td>
<td>70 GyE/10 Fx</td>
<td>99.2 Gy</td>
<td>95.2%</td>
<td>86.4%</td>
<td>73.9%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II (53%)</td>
<td>II (53%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III (11%)</td>
<td>III (11%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al. (2021)</td>
<td>Phase III</td>
<td>I (20%)</td>
<td>I (20%)</td>
<td>0%</td>
<td>66 GyE/10 Fx</td>
<td>91.9 Gy</td>
<td>88.9% (2 yr)</td>
<td>79.0% (4 yr)</td>
<td>PFS 20.9%</td>
<td>3–4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II (38%)</td>
<td>II (38%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III (41%)</td>
<td>III (41%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV (1%)</td>
<td>IV (1%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iwata et al. (2021)</td>
<td>Phase II</td>
<td>I (100%)</td>
<td>I (100%)</td>
<td>0%</td>
<td>66 GyE/10 Fx or 72.6 GyE/22 Fx</td>
<td>91.9 Gy</td>
<td>95% (2 yr)</td>
<td>84% (2 yr)</td>
<td>62% (2 yr)</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dose/Fx scheme, Dose/Fractionation scheme; EQD2, equivalent dose in 2 Gy fractions, using a linear quadratic model with a/b ratios of 10 for tumor; FFLP, free from local progression; OS, overall survival; IHF, intrahepatic failure; Rib Fx, rib fracture; RP, radiation pneumonitis; GIT, gastrointestinal toxicity; BCLC, Barcelona Clinic Liver Cancer; mUICC, modified Union for International Cancer Control.
of EBRT plus TACE with sorafenib in HCC with macroscopic vascular invasion, and demonstrated superior efficacy of EBRT plus TACE with survival improvement compared to systemic therapy. In neoadjuvant setting, EBRT provided significantly better postoperative survival outcomes in patients with resectable HCC and PVTT. Table 2 summarizes the results of previously published PBT data for HCC patients with vascular invasion. The reported median survival time of those patients after PBT was 16 to 22 months. These PBT outcomes were better than the outcomes expected of systemic treatment. In particular, the response rate was remarkably high, at up to 60–100%. After PBT, recanalization of the portal vein followed by the restoration of liver function might be a possible reason for the improvement in survival. In addition, treatment-related toxicity was relatively mild compared to systemic treatment, with ≥grade 3 toxicity rates of 0–13% after PBT. Among EBRT modalities for the treatment of HCC with macrovascular invasion, a recent meta-analysis showed a significantly better OS rate in the PBT group, which was 61%, 45%, and 45% in the PBT, conventional EBRT, and SBRT groups, respectively (P<0.05 for each comparison). For future perspectives, the combination of PBT with immune-checkpoint inhibitors such as anti-PD1/PDL1 is thought to be a promising treatment option, showing improved progression-free survival up to 27 months with curative intent treatment in a single retrospective study.

**CONCLUSION**

In conclusion, since PBT has a dosimetric benefit through superior physical properties, it has been thought to have advantages over conventional EBRT, as well as other local therapies, in the management of patients with HCC. Numerous clinical studies have demonstrated PBT as a highly effective local therapeutic option for early-to-advanced HCC, with favorable survival outcomes and a low toxicity rate. PBT is also being used successfully in challenging clinical conditions, such as major vascular invasion and re-irradiation cases. Nevertheless, the clinical evidence for PBT in HCC has been considered insufficient so far, as most studies may have involved inherent selection biases of a retrospective nature and biases towards new technologies, and it contains relatively few patients for high-level evidence. Hence, further research is warranted, and some studies are currently underway, including

<table>
<thead>
<tr>
<th>Author</th>
<th>Number</th>
<th>Tx modality</th>
<th>CPC (%)</th>
<th>Main VI (%)</th>
<th>RR (%)</th>
<th>2-yr FFLP (%)</th>
<th>Median OS (mo)</th>
<th>IHF (%)</th>
<th>Toxicity (≥Gr3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hata et al.</td>
<td>12</td>
<td>Proton</td>
<td>A (75)</td>
<td>B (25)</td>
<td>-</td>
<td>100</td>
<td>2-yr 88%</td>
<td>5-2y 58%</td>
<td>-</td>
</tr>
<tr>
<td>Sugahara et al.</td>
<td>60</td>
<td>Proton+/-TACE</td>
<td>A (80)</td>
<td>B (20)</td>
<td>-</td>
<td>100</td>
<td>2-yr 82%</td>
<td>5-2y 72%</td>
<td>-</td>
</tr>
<tr>
<td>Komatsu et al.</td>
<td>27</td>
<td>Proton</td>
<td>A (66.7)</td>
<td>B (33.3)</td>
<td>-</td>
<td>100</td>
<td>2-yr 82.8</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>27</td>
<td>Proton+/-Sorafenib</td>
<td>A (92.7)</td>
<td>B (7.3)</td>
<td>-</td>
<td>100</td>
<td>2-yr 82.9</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>41</td>
<td>Proton+/-Sorafenib</td>
<td>A (not reported)</td>
<td>B (not reported)</td>
<td>-</td>
<td>100</td>
<td>2-yr 88.1</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>13</td>
<td>Proton+/-Sorafenib</td>
<td>A (not reported)</td>
<td>B (not reported)</td>
<td>-</td>
<td>100</td>
<td>2-yr 88.1</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
<tr>
<td>Chadha et al.</td>
<td>29</td>
<td>Proton+/-anti-PD1/PDL1</td>
<td>A (100)</td>
<td>B (not reported)</td>
<td>-</td>
<td>100</td>
<td>2-yr 88.1</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
<tr>
<td>Sun et al.</td>
<td>29</td>
<td>Proton+/-anti-PD1/PDL1</td>
<td>A (100)</td>
<td>B (not reported)</td>
<td>-</td>
<td>100</td>
<td>2-yr 88.1</td>
<td>5-2y 69%</td>
<td>-</td>
</tr>
</tbody>
</table>

FFLP, free from local progression; OS, overall survival; IHF, intrahepatic failure; VI, vascular invasion; RR, response rate; NR, not reached; CPC, Child-Pugh classification.
comparisons of PBT with other treatment modalities or combinations, as well as other types of EBRT, such as photon EBRT or CIRT. For instance, comparisons of PBT versus ablative therapy in patients with treatment-naïve early-stage HCC, the efficacy of combinations of PBT and TACE or SIRT in intermediate-stage disease, or combinations of PBT and systemic therapy in advanced-stage disease will be promising research topics for future clinical trials and will enhance evidence-based clinical guidance and improve patient selection.

Authors’ Contribution
Conception, design of the study, literature review and analysis, critical revision and editing, and final approval of the final version: SU Lee and TH Kim.

Acknowledgements
This study supported by National Cancer Center grant (NCC-2110351).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


42. Nakayama H, Sugahara S, Fukuda K, Abei M, Shoda J, Sakurai


From nonalcoholic steatohepatitis, metabolic dysfunction-associated fatty liver disease, to steatotic liver disease: Updates of nomenclature and impact on clinical trials

Ming-Lun Yeh\textsuperscript{1,2} and Ming-Lung Yu\textsuperscript{1,2,3}

\textsuperscript{1}School of Medicine, College of Medicine, Kaohsiung Medical University; \textsuperscript{2}Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital; \textsuperscript{3}School of Medicine, College of Medicine and Center of Excellence for Metabolic Associated Fatty Liver Disease, National Sun Yat-sen University, Kaohsiung, Taiwan

\textbf{Keywords:} NAFLD; MAFLD; SLD; MASLD; Steatosis

With a growing prevalence, nonalcoholic fatty liver disease (NAFLD) has become the primary etiology of liver disease worldwide.\textsuperscript{1,2} However, the exclusionary diagnostic criteria raise concerns about using the term "NAFLD." In 2020, a panel of international experts from 22 countries proposed a new nomenclature of "metabolic dysfunction-associated fatty liver disease (MAFLD)" by a panel of experts in this field.\textsuperscript{3} As the name suggests, MAFLD emphasizes the importance of metabolic dysfunction that can be observed from the new definitions of overweight/obesity, type 2 diabetes, or at least two metabolic risk abnormalities, irrespective the etiologies and comorbidities, such as alcoholism and viral hepatitis. However, ignoring alcoholism and other specific etiologies raises concerns about the contributions of hepatic steatosis in the progression of liver disease and the stigmatization of the term "fatty". Recently, a new nomenclature, "Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD)," was set up by three pan-national liver associations to replace "NAFLD" and "MAFLD".\textsuperscript{4,5}

In the current issue of \textit{Clinical and Molecular Hepatology}, Kim et al.\textsuperscript{6} present their views regarding the potential impact of the new nomenclature "MASLD" on screening, diagnosis, treatment, and future drug development. Perspectives from hepatologists and endocrinologists were included as well. Unlike the negative criterion of NAFLD, MAFLD used a positive criterion and focused more on the linkage of metabolic abnormalities that were seen by the diagnostic criteria. More patients are diagnosed without the exclusion of other specific etiologies, and the disease awareness of physicians and patients has also improved. The most common cause of mortality in NAFLD patients was cardiovascular disease, followed by extrahepatic cancers.\textsuperscript{7} It was reported that MAFLD patients had a greater risk for all-cause mortality compared to...
NAFLD patients. Other reports also demonstrated an increased cardiovascular mortality of patients with MAFLD as compared to NAFLD. Similar results were also found in the risk of all types of cancers. That means the transition from NAFLD to MAFLD helps identify more subjects who are at risk of extrahepatic events, and further promotes the surveillance of extrahepatic diseases in clinical practice. Moreover, MAFLD also provides the opportunity to evaluate the interaction between NAFLD and hepatitis B virus (HBV) and hepatitis C virus (HCV).

However, as mentioned in this review, the abandonment of “steatohepatitis” disturbs the evaluation of hepatic severity and development of pharmaceutical agents. Meanwhile, the complete ignoring of alcohol consumption in MAFLD may confuse clinical judgment regarding the contributions of alcohol in hepatic progression. It is the same with the other specific etiologies that will also lead to hepatic steatosis and disease progression.

Different from NAFLD and MAFLD, the new term “Steatotic liver disease (SLD)” separates patients with or without cardiometabolic risk factors (CMRFs) and further classifies patients with CMRFs as “MASLD,” which indicates no specific etiology of steatosis, and “MetALD or other combined etiology” for those with a moderate amount of alcohol consumption or drug or monogenic disease-related steatosis. Those without CMRFs are categorized as “alcohol-related liver disease (ALD)” or “specific etiology SLD,” like drug-induced, monogenic, and miscellaneous, and “cryptogenic SLD” that not belong to the above categories. The new nomenclature “MASLD” also considers the hepatic progression form with a new term, “metabolic associated steatohepatitis (MASH),” which can be used as future guidance in clinical trials. The definition of alcohol amount is one of the points that differentiates SLD from NAFLD and MAFLD. Unlike the strict threshold of alcohol amounts in NAFLD and no threshold in MAFLD. A new category, “MetALD” is set up for those who consume moderate amounts of alcohol. The alcohol criteria of MetALD were made based on the general agreement that 30–60 gm of daily alcohol consumption would affect the natural history of NAFLD and possibly alter the response to therapeutic interventions. Recently, data from UK Biobank demonstrated that the MetALD group comprised predominantly males, and diabetes mellitus was significantly more prevalent in the MASLD group. The MetALD group also exhibited higher levels of liver enzymes but lower levels of high-density lipoprotein (HDL) cholesterol. The data implied the potential role of dyslipidemia in the pathogenesis and differentiation of MetALD and MASLD.

From the perspectives of hepatologists, both of the two new terminologies can increase disease awareness among patients and physicians. They are also expected to affect clinical practice positively, including the diagnostic process, non-pharmacologic approach, and potential treatment candidates. For clinical outcomes, the new subtypes of SLD might help identify more subjects at risk, either hepatic or extrahepatic. The new terminology operates subjects based on the amount of alcohol consumption that enables the development of proper treatment strategies accordingly and further helps to understand the influences of alcohol consumption in disease progression. Nevertheless, the criteria of alcohol consumption remain based on expert opinion and agreement, without scientific evidence. It is also difficult to assess alcohol consumption precisely in clinical practice.

The FDA recommends endpoints of clinical trials for accelerated approval of nonalcoholic steatohepatitis (NASH), including either improvement in steatohepatitis or fibrosis. Therefore, MAFLD is usually not included in the clinical trial enrollment criteria due to the lack of the term “steatohepatitis.” The new MASLD, with the progression form MASH, is expected to allow clinical trial enrollment. However, MASH also excludes NASH patients without CMRF from NASH treatment, although it might be rare. Whether the potential therapeutic agents for NASH could be generalized to MASH patients needs further investigation.

Despite that, several challenging issues of SLD remain.

1) There is difficulty in developing disease-specific biomarkers or agents for patients with MASLD, MetALD, and ALD. The dynamic changes in metabolic health and alcohol
consumption over time also raise the concern of making the diagnosis at a specific time. Currently, subjects who consume high amounts of alcohol together with metabolic dysfunction (positive CMRFs) are classified as ALD. However, this group of patients may have different disease pathogenesis, course, and outcomes than those without metabolic dysfunction.

2) Patients with HCV infection are classified as “miscellaneous SLD.” At least 20% of subjects have a spontaneous resolution from acute HCV infection, and most of the chronically infected patients are now eradicated owing to the current high-efficacy antiviral drugs. Chronic HCV infections are associated with the risks of extrahepatic manifestations, which frequently correlate to fatty liver, DM, cardiovascular comorbidities, even after HCV eradication. The role of metabolic dysfunction in the development of SLD before and after HCV eradication is clinically important, and it should not be excluded from clinical practice for SLD. Whether classifying it as HCV-SLD or HCV-MASLD may need further exploration.

3) HBV infection remains highly prevalent in middle- to old-aged Asians. However, HBV infection is not included in the new terminology regarding whether a specific classification of HBV should be made or not. Accumulating data have suggested that fatty liver and obesity facilitated higher chance of HBV surface antigen clearance and lower risk of cirrhosis and HCC in the natural course and during antiviral therapy. In contrast, coincidence of fatty liver and metabolic dysfunction increased the risk of hepatocellular carcinoma. Again, the interplay between HBV and SLD/MASLD should not be ignored in clinical practice.

From the perspectives of endocrinologists, the cardiometabolic risk threshold to determine metabolic dysfunction in SLD is discussed. As mentioned, using only one CMRF as the criteria may cause over-estimation of MASLD/MetALD and under-estimation of other types of SLD. Meanwhile, young and lean subjects with hepatic steatosis without any metabolic risk factors will be classified as cryptogenic SLD, even if they may share the same disease pathophysiology. Concerning the treatment of MASLD, therapeutic agents that are effective in metabolic syndrome may reverse MASLD. Same as for NAFLD/MAFLD, lifestyle modifications are the cornerstone, but are challenging for most patients. The glucose-lowering agents, sodium-glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide 1 (GLP 1) receptor agonists, have been shown to improve steatohepatitis and reduce cardiovascular risk. Thus, they may be applied in the treatment of MASLD, especially in those with type 2 diabetes. In the last part, the interaction between insulin resistance and alcohol consumption is discussed. However, the safe alcohol amount due to individual genetic differences and the relative contributions of metabolic dysfunction and alcohol to MetALD disease progression remain uncertain.

To conclude, Kim et al. reviewed the new terminology of SLD and its subclassifications, as well as the advantages and insufficiencies of the new terminology. As mentioned, future research is recommended for the new biomarkers and drugs for MASLD. Further explorations regarding the natural course and disease prognosis of the subtypes of SLD, especially MASLD, MetALD, and concomitant of viral hepatitis, are also necessary.

Authors’ contribution
ML Yeh drafted the manuscript. ML Yu reviewed and finalized the manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Adding to the confusion in more than just the name

Jacob George

Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Westmead, NSW, Australia

Keywords: MAFLD; Fatty liver disease; Metabolic steatohepatitis; MASLD

The year 2020 witnessed a paradigm shift in the way we conceptualised and thought about the fatty liver disease which is responsible for a majority of the cases we see in routine clinical practice. For the past 40 years, “non-alcoholic fatty liver disease (NAFLD)”, a term coined to define a histological lesion, was used to describe a disease entity that was clearly common and rising in prevalence in parallel with that of diabetes and overweight/obesity. Despite decades of discomfort with the term, NAFLD told clinicians and patients what the disease is not, instead of what the disease is, and was associated with the stigma linked to the term “alcohol”. Inertia, as is common in many areas of medicine, persisted. All this changed with two landmark papers by an international panel that proposed a new term, “metabolic (dysfunction) associated fatty liver disease” or metabolic dysfunction-associated fatty liver disease (MAFLD), and its new definition.1,2

What is not as well appreciated by the field is that the papers were a proposal firstly of a term that reflected accumulated knowledge on disease pathogenesis, and secondly and perhaps more importantly, it proposed a set of criteria on exactly what constituted the disease. The papers were published to wide acclaim (and some discontent) as a conceptual advance in the field, and were there for all clinicians to examine for its clinical utility at the bedside and for clinical research. As fatty liver disease due to metabolic dysregulation impacts the life-course, paediatric criteria were also proposed.3 Subsequent years have seen more than 4,000 citations for the two sentinel papers, over 7,000 publications using the MAFLD terminology and definition, and widespread acceptance in clinical practice guidelines including the first by the Asian Pacific Association for the Study of Liver (APASL),4 the Middle East and North Africa,5 the Chinese Society of Hepatology,6 and many other national societies as well as patient organisations.7 From the perspective of clinical research, MAFLD and its definition demonstrated clinical utility, increased disease awareness, and importantly, identified patients who are most at risk of hepatic and extrahepatic outcomes as compared to NAFLD.8-11 Another aspect that was not appreciated at inception was that MAFLD neatly stratified patients into three distinct groups (those with diabetes,
those with overweight/obesity, and those with MAFLD but a healthy weight), each with its own distinct patient profile in cross-sectional studies, and different disease trajectories and outcomes.1 Such stratification has allowed clinicians to prognosticate, and will enable tailored treatments based on phenotype in the future.

In the current issue, Kim and colleagues12 undertake an appraisal of the terminology and definition of another term, “metabolic dysfunction associated-steatotic liver disease (MASLD)”. Clearly, removal of any reference to alcohol in the proposed name is welcome and long overdue, as is acceptance of metabolic dysregulation as a core tenant and prerequisite for disease diagnosis. The fact that the proposal of MASLD has come after four decades highlights the inertia of societies and the importance of innovation and renewal from the grass roots in all scientific disciplines.

While the authors have undertaken an appraisal from both a hepatology and endocrinology perspective, as they imply, MASLD is a proposal with many unresolved questions. First and foremost, as suggested by others in the field and patient groups, the term “fatty liver”, when used to describe a liver with fat, is not stigmatising.13,14 Moreover, as circulated on social media and from first-hand experience, clinicians know from every day experience that when a patient is told they have MASLD, the first question asked is “what does steatotic liver disease mean,” to which the answer invariably is that you have a “fatty liver”.

Be that as it may, Kim and colleagues highlight that there are concerns with the MASLD definition, as well as several persisting misconceptions about the definition of MAFLD. It is stated that MAFLD fails to incorporate alcohol consumption into its diagnostic criteria. The simple answer to this often repeated statement, as highlighted in the original papers, is that MAFLD defines a particular form of liver disease due to systemic metabolic dysregulation; the disease (MAFLD) has nothing to do with whether or not a patient drinks alcohol, or for that matter, if the patient has concomitant viral hepatitis or not. For example, if a patient has hepatitis C, it does not mean that the patient cannot also have a second liver disease, such as hepatitis B. Only by defining what disease one is, can we decide if a patient also has disease two. MASLD fails to meet this basic tenant for disease diagnosis, which should encompass all patients with the disease. Using the MASLD terminology, if you meet the MASLD criteria and have “significant” alcohol consumption you have a different disease - MetALD. MetALD is not a separate disease but the co-existence of two concomitant diseases in the same person. By the MASLD logic, if a patient has hepatitis B infection or hepatitis C infection with MASLD, the patient should be given a separate disease name, as this is common in many parts of the world. MAFLD deftly avoids this issue by precisely defining what MAFLD is (similar to how we define what hepatitis C or B is) and stating that “patients who meet the criteria to diagnose MAFLD and who also have one of these concomitant conditions should be defined as having dual (or more) aetiology fatty liver disease”.2 In the example with a metabolic risk factor and hepatitis C, the steatotic liver disease (SLD) terminology reverts back to “combination aetiology”, exactly as proposed in the MAFLD definition. Identifying alcohol as a “special case” does not meet scientific scrutiny; as Kim and colleagues suggest, “the exact threshold for alcohol consumption that may lead to liver damage remains unclear. Although some studies have proposed protective effects of mild alcohol consumption others have indicated no safe level of alcohol consumption especially among individuals with MASLD. Furthermore, the extent of metabolic dysfunction and the amount of alcohol consumption may vary over time among individuals”.12 With these very real caveats, would it not be more consistent and logical to define each liver disease a person has on its own merits rather than adding a new disease term with an arbitrary definition? Even in those with alcohol consumption of >60 grams per day, it is a fallacy to consider that metabolic dysfunction will not contribute to their disease trajectory. In real life, liver disease outcomes are a combination of all the liver insults, however minor or major, and arbitrary categorisation simply muddies the water, something that MAFLD cannot be accused of.

Another common misconception is that of “oversight” of steatohepatitis. To be clear, MAFLD is a set of criteria for clinical diagnosis, while steatohepatitis is a histological diagnosis. The histological features of the disease (steatosis, steatohepatitis, and fibrosis) are what they are, and MAFLD in no way

---

**Abbreviations:**

NAFLD, nonalcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; APASL, Asian Pacific Association for the Study of Liver; MASLD, metabolic dysfunction associated steatotic liver disease; SLD, steatotic liver disease; HDL, high density lipoprotein; ALD, alcohol-related liver disease
detracts from the histological disease activity (metabolic steatosishepatitis) and/or fibrosis stage as reported in the original papers.1,2

The salient subcategories of SLD are illustrated in Figure 1; MASLD is diagnosed if one of the listed cardiometabolic risk factors are present in a person with hepatic steatosis. This would mean that a person with hypertension and hepatic steatosis has MASLD, or for that matter, steatosis and a low high density lipoprotein (HDL), with no clear evidence that these individuals have any adverse liver-related outcomes; for HDL and diastolic BP, their link to insulin resistance and steatosis is weak. A problem with the MASLD definition is that it tries to be “all things to all people,” which is a problem inherent in consensus (rather than data-driven) approaches. MASLD is exactly as per the previous NAFLD definition, a heterogeneous collection of diseases. Indeed, studies have suggested that MASLD and NAFLD are almost identical. In contrast, for MAFLD, several population-based studies have indicated that the three risk groups have varying initial presentations, different disease trajectories, and different hepatic and extrahepatic outcomes and in all cases, outcomes worse than those with NAFLD only, highlighting the clinical utility of the definition.8-11 As the critique suggests, over 90% of Koreans (and for that matter, people in most affluent countries) with SLD have at least one cardiometabolic risk factor. This “may lead to potential over-classification of MASLD and MetALD but under-classification of pure alcohol-related liver disease (ARLD), cryptogenic SLD, and SLD with specific aetiology.”12 Unlike the MAFLD criteria, which has a clear definition for MAFLD cirrhosis, the lack of a definition or a statement on MASLD-related cirrhosis also “continues to puzzle.”12

Kim and colleagues12 should be congratulated on their critique of MASLD. Given the various concerns, while MASLD is an advance on NAFLD, in many aspects it adds to confusion rather than representing a bold, innovative and rigorous attempt to redefine the field of fatty liver disease.

Conflicts of Interest

The author has no conflicts to disclose but there might be perceived conflicts as stated in ICJME form.

Advisory board of NovoNordisk, Gilead, Roche, Abbvie, Astrazeneca, Boehringer-Ingelheim.

REFERENCES

12. Kim GA, Moon JH, Kim W. Critical appraisal of metabolic dysfunction-associated steatotic liver disease: Implication of Janus-
Hepatocellular carcinoma (HCC) is usually developed in the background of chronic liver disease, including cirrhosis, the optimal treatment modality should be determined by tumor stage and baseline hepatic function as well. Although most HCC treatment guidelines suggest the recommended treatment for each stage, there are many cases in which alternative treatment options have to be selected for above-mentioned reason. Moreover, the major failure pattern is intrahepatic recurrences after initial treatment; however, there are no clear recommendations for the management of recurrent HCC. Due to the complexity of treatment decisions and involvement of multiple disciplines in the management of HCC, a multidisciplinary approach should be considered to improve outcomes of HCC patients.

Historically, external beam radiotherapy (RT) had a very limited role in the treatment of HCC, due to the uncertain treatment planning, beam delivery, and a consequence of deterioration of hepatic function even after limited doses, RT had been used in some patients with extrahepatic lesions for symptom palliation. However, recent improvement of computer technology into radiotherapy techniques, including three-dimensional conformal RT, intensity-modulated RT, 4-dimensional computed tomography with strategies for respiratory motion management, and image-guided RT has allowed a more precise treatment to enhance the efficacy and minimize treatment-related complications. Therefore, the role of RT as an alternative treatment method in the management of HCC has steadily increased, and its effectiveness has been emphasized more recently. According to the recent publications, stereotactic body RT, which refers to the delivery of high doses of radiation to the tumor in few fractions, offers potentially curative local therapy for patients with small HCC who are not candidates for hepatic resection or thermal ablation. In addition, RT, combined with transarterial chemoembolization or other locoregional therapies, can
be used as a first-line treatment for patients with advanced HCC showing macroscopic vascular invasion for better progression-free and overall survival rates.\textsuperscript{9,10}

In addition to the development of RT using X-ray (photon) as mentioned above, the use of particle therapy for treating HCC is also a new technical advance among the RT methods. Particle therapy that can be used in the management of HCC is mainly divided into proton beam therapy (PBT) and carbon ion RT (CIRT).\textsuperscript{11,12} The main physical characteristics of particle therapy is the absorbed dose curve shows a slow initial increase with the penetration through matter, than a steep rise to the maximum, known as the Bragg peak, followed by a sharp fall in energy absorption toward the end of the beam range.\textsuperscript{13} This unique characteristics can be able to provide a more precise dose delivery to the tumor, while minimizing radiation effect to the surrounding normal tissues effectively. The potential benefits of particle therapy, particularly in HCC, may be grounded in the following rationales: HCC mainly occurs as a consequence of pre-existing liver cirrhosis, which imposes limitations on remnant hepatic volume compared with non-cirrhotic healthy liver. In addition, since intrahepatic recurrence is frequent, it is necessary to preserve the uninvolved liver as much as possible during one treatment session to help determine the following treatment at the time of recurrence. In this issue of Clinical and Molecular Hepatology, Lee and Kim\textsuperscript{12} demonstrated that PBT was a highly effective local therapeutic option for early-to-advanced stage HCC, with favorable survival outcomes and a low toxicity rate. They also mentioned PBT had been used successfully in challenging clinical conditions, such as major vascular invasion and re-irradiation.\textsuperscript{17} Recently, the first phase III randomized controlled trial demonstrated that PBT was associated with local progression-free survival rates that were comparable to those observed for thermal ablation in patients with recurrent small (<3 cm) HCC.\textsuperscript{14}

In addition to aforementioned advantage of particle therapy, CIRT possesses further distinctive attributes. The heavy charged ions have less lateral scattering than other particles; therefore, the degree of lateral penumbra for CIRT is smaller than that of PBT.\textsuperscript{15} Moreover, CIRT has biological effectiveness which is detailed in another review article in the current issue of Clinical and Molecular Hepatology.\textsuperscript{11} To summarize briefly, carbon ions are high linear energy transfer (LET) radiations and high-LET radiation tends to have a higher relative biological effectiveness (RBE) than low-LET radiations. The local RBE values for carbon ions can reach as high as 2.0–3.5 depending on their position within the treatment beam (RBE of X-rays: 1, RBE of PBT: 1.1).\textsuperscript{15} Through these biological advantages, CIRT can offer clinical benefits in controlling hypoxic or radioresistant tumors.\textsuperscript{11,14} As CIRT is a relatively new approach and is installed in a few institutions, there are limited well-controlled clinical trials, including for HCC. Further studies are necessary to provide robust clinical evidence of CIRT in treating HCC in the future.

The development of RT techniques in treating HCC undoubtedly holds the potential to enhance therapeutic efficacy and broaden its applicability. However, several points must be considered for future research. Firstly, there is a need for answers regarding the clinical scenarios in which particle therapy would provide more benefits compared with RT using X-rays. More comprehensive dosimetric studies along with the consensus reports from the expert could contribute to making an informed decision.\textsuperscript{17} Secondly, while a higher RBE could enhance treatment outcomes for tumors, it is necessary to investigate whether the uncertain RBE might lead to increased toxicities in surrounding organs, including the uninvolved liver especially for CIRT. Lastly, research pertaining to cost-effectiveness should also be undertaken.

In summary, with the progress of cutting-edge technology, RT has emerged as a safe and more efficacious modality for treating HCC. As more clinical experiences and research on particle therapy unfold in the future, it is anticipated that better outcomes might be achieved for patients with HCC, surpassing the current standards.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

1. Marrero JA, Kulik LM, Sirin CB, Zhu AX, Finn RS, Abecassis MM,


Lean or non-obese nonalcoholic fatty liver disease patients: Are they really lean?

Eugene Han¹ and Yong-ho Lee²,³

¹Department of Internal Medicine, Keimyung University School of Medicine, Daegu; ²Department of Internal Medicine, Yonsei University College of Medicine, Seoul; ³Institute of Endocrine Research, Yonsei University College of Medicine, Seoul, Korea

Keywords: Nonalcoholic fatty liver disease; Body mass index; Obesity; Sarcopenia; Body composition

The prevalence of nonalcoholic fatty liver disease (NAFLD) has dramatically increased in recent decades, parallel with the expansion of the obese population.¹ However, a substantial number of individuals with NAFLD are lean. The proportion of lean or non-obese and obese NAFLD patients among those with NAFLD is 19.2% and 40.8%, respectively.² The definition of lean or non-obese is based on the body mass index (BMI) with ethnic-specific cut-off points. Given the lower prevalence of NAFLD in the Asian countries compared to that in Western countries and the comparable proportion of lean or non-obese proportion of NAFLD between those populations,² the Asian population, with a relatively lower BMI, is more vulnerable to NAFLD. Moreover, Asians tend to have more visceral fat deposition with a similar BMI compared to other ethnicities.³ Therefore, lean or non-obese NAFLD was considered a unique phenomenon among Asians in early studies, which is why most existing studies were conducted in Asian countries.²

In this issue of Clinical and Molecular Hepatology, Kim et al.⁴ demonstrated that visceral fat obesity had the strongest association with non-obese NAFLD, the impact of which is greater than that of diabetes or systemic inflammation. Although the pathophysiological mechanisms of non-obese or lean NAFLD are unclear, growing evidence supports that visceral obesity rather than BMI-based obesity plays a crucial role.⁵ The association between sarcopenia, myosteatosis, and NAFLD is based on insulin resistance,⁶,⁷ and myosteatosis rather than sarcopenia determines the early stage of NAFLD progression.⁸ Given that lipid oxidation capacity regulated by visceral adiposity controls lipid accumulation in muscle,⁹,¹⁰ visceral adiposity might be the main and early phenotype of lean or non-obese NAFLD individuals as well as obese NAFLD individuals. Therefore, the BMI-based obesity index does not accurately reflect visceral adiposity or obesity. Kim et al.⁴ reported no statistical difference in the visceral to subcutaneous fat ratio between non-obese and obese NAFLD patients in the male population, although the values of those with NAFLD were higher than those without NAFLD. Another Korean population-based study reported that visceral adiposity was the main contributor to incident NAFLD¹¹ with a baseline mean BMI of 23.7 kg/m², indicating that most individuals...
were non-obese; however, the mean waist circumference was 85.1 cm. Considering the sex ratio (40.2% female) and waist circumference cut-off value for central obesity in Asian women (85 cm), a substantial number of individuals were centrally obese with a normal BMI.

NAFLD and nonalcoholic steatohepatitis are well-established risk factors for cardio-renal and metabolic diseases as well as advanced liver diseases, such as cirrhosis and cancers. Although some data suggest that lean NAFLD individuals have less-severe disease and may have a more favorable outcome than their obese counterparts, lean NAFLD individuals also experienced both hepatic and extrahepatic complications. The histological severity in non-obese NAFLD compared to obese counterparts seemed similar in terms of lobular inflammation and ballooning. In longitudinal biopsy-proven NAFLD studies, the mortality risk of lean or non-obese NAFLD patients is similar to that of obese NAFLD patients. A Chinese study demonstrated a better outcome in non-obese NAFLD patients compared to obese NAFLD patients, where the authors enrolled more individuals with liver fibrosis (55.1% vs. 80.1% for non-obese NAFLD vs. obese NAFLD, respectively) or those with metabolic syndrome (43.1% vs. 69.8% for non-obese NAFLD vs. obese NAFLD, respectively) in the obese group. The follow-up duration was relatively short (median 49 months) for determining mortality. Nevertheless, the results indicated that risk factors such as hypertriglyceridemia and increased creatinine levels were associated with NAFLD progression in lean patients, suggesting that dysregulated metabolic profiles are key to disease prognosis. In this context, metabolic dysregulation, instead of BMI-based obesity, may better reflect the clinical outcomes of NAFLD. Lean NAFLD individuals with metabolically unhealthy profiles have a higher risk for cardiovascular disease than obese NAFLD individuals. Likewise, the association between NAFLD and sarcopenia was strengthened in metabolic dysfunction. In terms of sarcopenia as one of the predisposing phenotypes of NAFLD, the association between sarcopenia and NAFLD appears in both the obese and non-obese NAFLD populations, even though Kim et al. did not report a statistically significant association between sarcopenic obesity and NAFLD, probably due to the small sample size of sarcopenic individuals with higher BMI. When NAFLD is combined with sarcopenia, the risk of cardiovascular disease increases regardless of obesity. The above evidence suggests the need for a new index to predict cardiometabolic risk other than BMI.

In terms of NAFLD management, especially in lean populations, Kim et al. provided clinical implications for body composition as a treatment goal. Reducing visceral fat and improving muscle quality and mass, instead of reducing body weight, were the main strategies for NAFLD treatment. In a Korean study with a median 4.4-year follow-up, NAFLD was resolved in 24.7% of individuals, whose visceral adipose tissue amounts were significantly reduced, indicating a close relationship between visceral adiposity and NAFLD risk. Physical activity is recommended as it improves insulin resistance in adipose tissue, liver, and skeletal muscle, the triad in NAFLD development, and improved muscle function further ameliorates systemic insulin resistance, and hepatic inflammation can be resolved. Exercise can ameliorate the risks of NAFLD and liver fibrosis, even in individuals with sarcopenia. Additionally, physical exercise improves cardiopulmonary fitness, which can decrease cardiovascular events and mortality. Although there are limited data on the type or duration of exercise on NAFLD regression, moderate to vigorous exercise might be helpful. A cross-sectional study showed that physical activity over 880 metabolic equivalent tasks/min/week decreased the risk of fibrosis, sarcopenia, and cardiovascular disease.

In summary, the study by Kim et al. supported the evidence that visceral obesity is a risk factor for sarcopenia and/or myosteatosis in non-obese NAFLD individuals. This result was consistent with that of NAFLD as an ectopic fat deposit that manifested as visceral obesity, sarcopenia, and myosteatosis. Although the term “lean or non-obese” NAFLD individuals is widely accepted, the natural history or clinical prognosis of lean or non-obese NAFLD patients is comparable to that of obese NAFLD patients. Therefore, the BMI-based obesity index may need to be revised, and metabolic dysfunction should be considered to identify high-risk individuals with NAFLD. Moreover, NAFLD treatment strategies, besides reducing body weight, should be established to im-

Abbreviations:
NAFLD, nonalcoholic fatty liver disease; BMI, body mass index

prove body composition, which includes the reduction of visceral adipose tissues and the increment of muscle mass/quality.

Authors’ contribution
All authors were responsible for the conceptualization, interpretation of data, drafting, and critical revision of the manuscript.

Acknowledgements
National Research Foundation grant funded by the Korea Government (MSIP). Grant Numbers: 2021R1G1A1010975.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Chemoembolization combined with radiofrequency ablation is the best option for the local treatment of early hepatocellular carcinoma?

Hyo-Cheol Kim
Department of Radiology, Seoul National University Hospital, Seoul, Korea

Keywords: Hepatocellular carcinoma; Chemoembolization; Radiofrequency ablation

This study presents a valuable contribution to the field of hepatocellular carcinoma (HCC) treatment, addressing the ongoing debate surrounding the most effective nonsurgical approaches for early stage HCC. The researchers conducted a network meta-analysis to compare the efficacy of various locoregional treatments, and their results elucidated the relative ranking of these interventions. Furthermore, chemoembolization combined with radiofrequency ablation (RFA) demonstrated superior overall survival (OS) and overall progression-free survival (PFS) compared with RFA alone.

This study indicates that combined RFA and chemoembolization therapy is the most effective option for the local treatment of early HCC; however, acknowledging the potential shortcomings and limitations associated with this treatment approach is essential.

First, successful combined therapy relies primarily on appropriate patient selection. This treatment is not suitable for all patients with early stage HCC because of various factors such as tumor location, size, and liver function. Tumor location is the most critical factor for determining the treatment modality, which is seldom considered in the literature, from the viewpoint of interventional radiologists. Cryoablation may be safer than radiofrequency ablation when the tumor is located near the gallbladder or bowel. Microwave ablation, cryoablation, or external radiation therapy may be more effective than radiofrequency ablation when the tumor abuts the large vessels. Deep-seated tumors in the Spiegel lobe may be challenging for percutaneous ablation but can be easily treated with superselective chemoembolization or radioembolization with cone-beam computed tomography guidance. A tumor just below the heart may be more suitable for intra-arterial than for percutaneous therapy. A 3-cm tumor size is a crucial number in treatment triage, and most Asian guidelines recommend local ablation for HCCs ≤3 cm. Similarly, radiofrequency ablation is seldom performed in patients with tumors >3 cm at our institute. However, the inclusion criterion in this study was a tumor size of <5 cm. Tumors 3–5 cm in size may benefit more from the combined therapy of ablation and chemoembolization. In particular,
combined ablation and chemoembolization therapy may not be superior to ablation alone in patients with tumors measuring <3 cm. Most patients with Child-Pugh class A are unlikely to experience severe liver function impairment after combined ablation and chemoembolization. However, some patients with Child-Pugh class B/C may be susceptible to overtreatment.

Second, the landscape of HCC treatment is continually evolving with the introduction of novel therapies and technological advancements, including no-touch ablation and radioembolization. The data collection period of this study may not fully account for the most recent developments, and newer treatment options may have emerged that could challenge or complement the effectiveness of the combined therapy. In particular, the no-touch ablation technique has demonstrated excellent local tumor control, with a reduction in the one-year local recurrence rate by approximately one-third. Additional chemoembolization is no longer required in most cases of ablation according to the operator’s decision because no-touch ablation has been routinely attempted in our institutes recently. Additionally, radioembolization with curative intent for early stage HCC, which was deemed unfavorable for ablation, demonstrated excellent tumor response at a high medical cost.

Third, the combined therapy of RFA and chemoembolization may significantly increase medical costs owing to prolonged hospitalization and increased medical resource usage. Combined therapy is not fully reimbursed in some countries owing to increased medical costs and insufficient evidence of its superiority over monotherapy. The economic burden of such treatment and its impact on healthcare resources when assessing its overall value and feasibility should be considered because the current study revealed improved PFS over monotherapy, thereby decreasing the requirement of subsequent therapy and reducing overall medical costs in the long-term follow-up period.

Fourth, the combination of RFA and chemoembolization represents a complex treatment regimen that involves multiple interventions and requires expertise in both the techniques. This complexity may increase the risk of procedural complications and adverse events, although many studies have reported that combination treatment does not increase serious complications. The study may have not fully addressed the safety profile and potential complications associated with this combined approach, thereby warranting further investigation to assess its risk–benefit ratio. Additionally, doctors may not consider manageable pain to be significant, but most patients desire to receive treatment comfortably. The combination of RFA and chemoembolization seems natural to involve more discomfort for patients than monotherapy, which may not be considered in most studies.

The study also emphasizes that cryo, microwave, and laser ablation, and proton beam therapy have similar effects on OS as RFA. This information is valuable for clinicians when considering alternative treatment options for patients who may not be suitable candidates for a combination of chemoembolization and RFA.

However, the results revealed the relative ineffectiveness of percutaneous ethanol or acetic acid injection compared with RFA for all measured outcomes. This finding underscores the need for caution when considering these modalities and indicates that this is not the optimal choice for early HCC treatment. Despite the strengths of this study, some limitations should be acknowledged. First, the inclusion of only randomized trials could potentially limit the generalizability of the findings because real-world patient populations may differ. Second, the availability of studies on certain interventions may have been limited, thereby potentially affecting the robustness of the network meta-analysis. Moreover, the evolving landscape of HCC treatment should be considered because new therapies and techniques may have emerged since the data collection period.

In conclusion, this study provides valuable insights into the comparative efficacy of various nonsurgical treatments for early HCC. These findings revealed that chemoembolization combined with RFA is the most effective option for local treatment, which may have significant clinical implications. However, further research, including head-to-head randomized trials and investigations of newer treatments, is warranted to validate and refine these findings. Clinicians should carefully consider these results when deciding on early stage therapy.

Abbreviations:
HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; OS, overall survival; PFS, progression-free survival

HCC treatment and tailor their approach based on individual patient characteristics and contraindications for specific treatments.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

Association of visceral fat obesity, sarcopenia, and myosteatosis with non-alcoholic fatty liver disease without obesity

Hong-Kyu Kim1*, Sung-Jin Bae1*, Min Jung Lee1, Eun Hee Kim1, Hana Park2, Hwi Seung Kim3,4, Yun Kyung Cho3,4, Chang Hee Jung3,4, Woo Je Lee3,4, and Jaewon Choe3

1Subdivision of Endocrinology and Metabolism, Health Screening and Promotion Center, Asan Medical Center, Seoul; 2Subdivision of Gastroenterology and Hepatology, Health Screening and Promotion Center, Asan Medical Center, Seoul; 3Division of Endocrinology and Metabolism, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul; 4Asan Diabetes Center, Asan Medical Center, Seoul, Korea

Graphical Abstract

In addition to visceral fat obesity (VFO), both sarcopenia and myosteatosis were significantly associated with non-alcoholic fatty liver disease (NAFLD) in non-obese individuals.

Study Highlights

- In addition to visceral fat obesity, both sarcopenia and/or myosteatosis were significantly associated with NAFLD in non-obese individuals.
- These results suggest that improvement of body composition, including reducing visceral adipose tissue, increasing skeletal muscle mass, and improving myosteatosis, should be considered for managing NAFLD in non-obese individuals.
INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has long been a leading cause of morbidity due to chronic liver disease in Western countries, and the prevalence of NAFLD is also increasing in Asian countries. Although NAFLD is commonly observed in individuals with obesity, many epidemiologic data showed that NAFLD may also be present in non-obese individuals, suggesting that factors other than obesity contribute to the development of NAFLD.

Several studies have reported that the contribution of visceral fat or visceral fat obesity (VFO) to NAFLD is more important.
tant than general measures of obesity represented by body mass index (BMI) or total body fat. Individuals with NAFLD have more visceral5,6 or visceral to subcutaneous fat ratio (VSR)8 than individuals without NAFLD.

Sarcopenia has been shown to be associated with NAFLD and its complications such as non-alcoholic steatohepatitis (NASH) and liver fibrosis independent of obesity. However, there have been only few studies about the association between sarcopenia and NAFLD, which reported inconsistent results according to body size adjustment such as height, weight, and BMI.9,10,15

Myosteatosis refers to ectopic fat infiltration into skeletal muscles including intramyocellular lipid and intermuscular fat and is known to be associated with insulin resistance and muscle dysfunction.11,12 We have previously observed that myosteatosis plays an important role in the association between skeletal muscle mass and cardiometabolic diseases and studied various measurements of skeletal muscle mass and calculated the indices for myosteatosis obtained by computed tomography (CT) scan at the 3rd lumbar vertebral level.

These previous studies led us to investigate which body compositional characteristics are associated with non-obese NAFLD. Therefore, the purposes of this study were (1) to confirm that non-obese individuals with NAFLD have more visceral adipose tissue (VAT) and/or higher VSR than non-obese individuals without NAFLD, (2) to investigate whether low skeletal muscle mass and/or degree of myosteatosis is independently associated with non-obese NAFLD, and (3) to compare the prevalence of VFO, sarcopenia, and/or myosteatosis between non-obese individuals with NAFLD and those without.

MATERIALS AND METHODS

Study population

We performed a cross-sectional study on 23,311 individuals aged 20 years or older who underwent abdominal CT scans during routine health examinations at the Health Screening and Promotion Center of Asan Medical Center (Seoul, Korea) between January 2012 and December 2013. Detailed information about this study population, laboratory measurements, anthropometric and body composition measurements, and CT image acquisition, and statistical analysis are provided in Supplementary materials.

The study protocol was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2018-0917), which provided an exemption of written informed consent because this is a retrospective analysis of pre-existing clinical data that were de-identified before the analysis and had therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Definitions of NAFLD and liver fibrosis

NAFLD was diagnosed with hepatic ultrasonography (Ultrasound Systems IU22; Philips, Best, The Netherlands) by expert radiologists. Fatty liver was diagnosed according to characteristic ultrasonographic findings, such as parenchymal brightness, liver-to-kidney contrast, blurring vessels, focal sparing, and narrowing of the lumen of the hepatic veins. Fatty liver severity was classified as non-fatty liver, mild, moderate, or severe fatty liver according to the findings of the bright liver, hepatorenal echo contrast, blurring of vessels, and deep attenuation of the ultrasound signal. Hepatic steatosis was defined by the fatty liver index (FLI): FLI ≥30.25 The fibrosis-4 index (FIB-4) was calculated only in individuals with NAFLD, and significant liver fibrosis was defined as FIB-4 >2.67, which has shown good diagnostic performance for detecting significant liver fibrosis.26

Assessment of skeletal muscle area and quality

Body composition was evaluated with abdominal CT using an automated artificial intelligence software developed using a fully convolutional network segmentation technique. The software automatically selects axial CT slices at the L3 vertebrae inferior endplate level. Then, the selected CT images are automatically segmented to generate boundaries of total abdominal muscle area (TAMA), visceral fat area (VFA), and subcutaneous fat area (SFA). For muscle quality evaluation, the TAMA was divided into three areas according to the CT density as follows: (1) inter/intra-muscular adipose tissue (IMAT, −190 to −30 Hounsfield units; HU), reflecting the apparent fat tissue between muscle groups and muscle fibers, (2) normal attenuation muscle area (NAMA, +30 to +150 HU), reflecting healthy muscle with little intramuscular fat, and (3)
low attenuation muscle area (LAMA, −29 to +29 HU), reflecting unhealthy muscles with intramuscular lipid pool. The skeletal muscle area (SMA, −29 to +150 HU) referred to the combined area of the NAMA and LAMA. All measurements were adjusted by the square of the height (m²), weight (kg), or BMI. The NAMA/TAMA index was calculated by dividing the NAMA by TAMA and multiplying by 100.

Definitions of generalized obesity, visceral fat obesity, sarcopenia, and myosteatosis

Obesity (BMI ≥25 kg/m²) and non-obesity (BMI <25 kg/m²) were defined according to the Asia-Pacific criteria established by the World Health Organization Western Pacific Region. VFO was defined by the visceral-to-subcutaneous ratio (VSR) (VSR≥1.0 in men; VSR≥0.5 in women). VSR was calculated by dividing VFA by SFA. Sarcopenia was defined as BMI-adjusted SMA below one standard deviation (SD) from the sex-specific mean value for the healthy young population (20–44 years). Additional analysis with sarcopenia defined by height-adjusted SMA is shown in the Supplementary data. Myosteatosis was defined by a T-score less than −1.0 of the NAMA/TAMA index (<73 in men; <72 in women).

RESULTS

Clinical characteristics of study participants

A total of 14,400 individuals (7,470 men and 6,930 women) were included in the analysis. The mean age was 53.5±9.0 years. Supplementary Table 1 shows the summary of the clinical characteristics of the study individuals according to sex. Men and women were significantly different in all variables including anthropometric measurements, body composition parameters, lifestyle factors, and prevalence of diabetes and hypertension; therefore, statistical analyses were performed separately in each sex.

Prevalence of NAFLD according to the presence of obesity

Of the 14,400 individuals, 4,748 (33.0%) had NAFLD (42.0% in men and 23.2% in women). Among 4,748 individuals with NAFLD, 2,161 (45.5%) were non-obese (40.7% in men and 54.8% in women). The prevalence of NAFLD in non-obese and obese individuals was 21.4% (28.0% in men and 15.9% in women) and 60.4% (64.0% in men and 52.9% in women), respectively. When NAFLD was categorized into three subgroups based on the severity by the sonographic findings, 61.1% of cases in the individuals with NAFLD were catego-
Table 1. Comparison of CT measurements between those with NAFLD and those without according to the presence of obesity in men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without obesity</th>
<th>With obesity</th>
<th>P-value</th>
<th>Without obesity</th>
<th>With obesity</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>3,286 (44.0)</td>
<td>1,278 (17.1)</td>
<td>0.650</td>
<td>1,046 (14.0)</td>
<td>1,860 (24.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>54.2±9.4</td>
<td>54.1±8.4</td>
<td>0.660</td>
<td>54.0±9.4</td>
<td>52.3±9.0**</td>
<td>0.026</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.4±5.7</td>
<td>170.3±5.5</td>
<td>&lt;0.001</td>
<td>170.4±5.9</td>
<td>170.9±5.8**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.0±6.7</td>
<td>68.2±5.5</td>
<td>&lt;0.001</td>
<td>76.9±7.0**</td>
<td>80.3±9.1**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4±1.7</td>
<td>23.5±1.1</td>
<td>&lt;0.001</td>
<td>26.5±1.4**</td>
<td>27.5±2.2**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>81.6±5.7</td>
<td>85.9±4.2</td>
<td>&lt;0.001</td>
<td>91.2±5.3**</td>
<td>94.6±6.3**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>18.5±4.2</td>
<td>21.6±3.5</td>
<td>&lt;0.001</td>
<td>23.9±4.1**</td>
<td>25.9±4.5**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM (kg)</td>
<td>22.6±2.7</td>
<td>22.8±2.5</td>
<td>0.170</td>
<td>24.8±2.7**</td>
<td>25.2±3.0**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM/height (kg/m²)</td>
<td>7.77±0.59</td>
<td>7.82±0.49</td>
<td>0.026</td>
<td>8.53±0.51**</td>
<td>8.62±0.59**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM/weight (%)</td>
<td>34.8±2.4</td>
<td>33.4±1.9</td>
<td>&lt;0.001</td>
<td>32.3±1.8**</td>
<td>31.5±2.0**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM/BMI</td>
<td>1.01±0.12</td>
<td>0.97±0.10</td>
<td>&lt;0.001</td>
<td>0.94±0.10**</td>
<td>0.92±0.10**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat measurements by CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>97.6±34.1</td>
<td>113.9±26.8</td>
<td>&lt;0.001</td>
<td>145.4±41.3**</td>
<td>161.6±53.8**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>89.5 (57.4, 119.6)</td>
<td>136.9 (112.2, 164.3)</td>
<td>&lt;0.001</td>
<td>149.8 (119.7, 184.5)**</td>
<td>182.6 (153.4, 221.8)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/height (cm²/m²)</td>
<td>30.5 (19.7, 41.8)</td>
<td>46.9 (38.5, 56.9)</td>
<td>&lt;0.001</td>
<td>51.6 (41.1, 63.8)**</td>
<td>63.2 (52.4, 76.0)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/weight (cm²/kg)</td>
<td>1.36 (0.91, 1.80)</td>
<td>1.99 (1.64, 2.41)</td>
<td>&lt;0.001</td>
<td>1.96 (1.56, 2.41)</td>
<td>2.31 (1.95, 2.76)**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/BMI</td>
<td>0.88 (0.65, 1.16)</td>
<td>1.19 (0.96, 1.53)</td>
<td>&lt;0.001</td>
<td>1.06 (0.81, 1.36)**</td>
<td>1.20 (0.93, 1.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VSR</td>
<td>0.88 (0.65, 1.16)</td>
<td>1.19 (0.96, 1.53)</td>
<td>&lt;0.001</td>
<td>1.06 (0.81, 1.36)**</td>
<td>1.20 (0.93, 1.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Muscle measurements by CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAMA (cm²)</td>
<td>156.9±18.7</td>
<td>160.4±16.1</td>
<td>&lt;0.001</td>
<td>178.9±19.8**</td>
<td>183.7±20.7**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMA/height (cm²/m²)</td>
<td>54.1±6.0</td>
<td>55.3±5.2</td>
<td>&lt;0.001</td>
<td>61.6±6.0**</td>
<td>62.9±6.5**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMA/weight (cm²/kg)</td>
<td>2.42±0.22</td>
<td>2.38±0.19</td>
<td>&lt;0.001</td>
<td>2.33±0.22**</td>
<td>2.30±0.19**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMA/BMI</td>
<td>70.2±0.69</td>
<td>68.3±0.62</td>
<td>&lt;0.001</td>
<td>6.76±0.69**</td>
<td>6.70±0.66**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA (cm²)</td>
<td>152.4±18.5</td>
<td>155.3±16.1</td>
<td>&lt;0.001</td>
<td>172.5±20.0**</td>
<td>176.8±20.2**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA/height (cm²/m²)</td>
<td>52.5±6.0</td>
<td>53.6±5.2</td>
<td>&lt;0.001</td>
<td>59.4±6.2**</td>
<td>60.5±6.4**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA/weight (cm²/kg)</td>
<td>2.35±0.22</td>
<td>2.28±0.19</td>
<td>&lt;0.001</td>
<td>2.25±0.23**</td>
<td>2.21±0.22**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA/BMI</td>
<td>6.82±0.71</td>
<td>6.62±0.62</td>
<td>&lt;0.001</td>
<td>6.52±0.71**</td>
<td>6.22±0.22**</td>
<td>0.009</td>
</tr>
<tr>
<td>NAMA (cm²)</td>
<td>125.5±19.6</td>
<td>126.1±18.3</td>
<td>0.370</td>
<td>135.0±22.7**</td>
<td>138.3±22.1**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAMA/height (cm²/m²)</td>
<td>43.2±6.5</td>
<td>43.5±6.1</td>
<td>0.250</td>
<td>46.5±7.6**</td>
<td>47.4±7.4**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Variable</td>
<td>Without obesity</td>
<td>With obesity</td>
<td>P-value</td>
<td>Without obesity</td>
<td>With obesity</td>
<td>P-value</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>NAMA/weight (cm²/kg)</td>
<td>1.94±0.28</td>
<td>1.85±0.25</td>
<td>&lt;0.001</td>
<td>1.76±0.29''</td>
<td>1.73±0.29''</td>
<td>0.000</td>
</tr>
<tr>
<td>NAMA/BMI</td>
<td>5.62±0.86</td>
<td>5.37±0.76</td>
<td>&lt;0.001</td>
<td>5.11±0.86''</td>
<td>5.06±0.84''</td>
<td>0.120</td>
</tr>
<tr>
<td>IMAT (cm²)</td>
<td>3.5 (2.2, 5.6)</td>
<td>4.0 (2.6, 6.4)</td>
<td>&lt;0.001</td>
<td>5.1 (3.3, 8.2)</td>
<td>5.3 (3.5, 8.5)</td>
<td>0.130</td>
</tr>
<tr>
<td>LAMA (cm²)</td>
<td>25.8 (20.6, 31.8)</td>
<td>28.0 (23.3, 34.0)</td>
<td>&lt;0.001</td>
<td>35.6 (29.1, 43.5)''</td>
<td>36.3 (29.7, 44.5)''</td>
<td>0.068</td>
</tr>
<tr>
<td>NAMA/TAMA index</td>
<td>79.6±7.1</td>
<td>78.4±6.8</td>
<td>&lt;0.001</td>
<td>75.3±8.1''</td>
<td>75.2±8.3''</td>
<td>0.850</td>
</tr>
<tr>
<td>FRI</td>
<td>17.8±12.7</td>
<td>31.6±15.7</td>
<td>&lt;0.001</td>
<td>41.3±18.2''</td>
<td>56.6±19.4''</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FIB-4</td>
<td>-</td>
<td>1.18 (0.90, 1.50)</td>
<td>&lt;0.001</td>
<td>-</td>
<td>1.15 (0.89, 1.48)</td>
<td>0.114</td>
</tr>
<tr>
<td>FIB-4 &gt;2.67 (%)</td>
<td>-</td>
<td>2.0</td>
<td></td>
<td>-</td>
<td>1.8</td>
<td>0.791</td>
</tr>
</tbody>
</table>

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

NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; PBF, percent body fat; ASM, appendicular skeletal muscle mass; CT, computed tomography; VFA, visceral fat area; VSR, visceral to subcutaneous fat ratio; TAMA, total abdominal muscle area; SMA, skeletal muscle area; IMAT, inter/intra-muscular adipose tissue; LAMA, low attenuation muscle area; NAMA, normal attenuation muscle area; NAMA,TAMA index=(NAMA/TAMA)×100; FLI, fatty liver index; FIB-4, fibrosis-4 index.

P-values represent the comparison between the groups with and without NAFLD by paired t-test or Mann–Whitney U-test for continuous variables and by chi-square test for categorical variables. Asterisk (*) denotes comparison vs. NAFLD without obesity group (*P<0.05; **P<0.001).
Table 2. Comparison of CT measurements between those with NAFLD and those without according to the presence of obesity in women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without obesity</th>
<th>NAFLD</th>
<th>P-value</th>
<th>With obesity</th>
<th>NAFLD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>4,673 (67.4)</td>
<td>883 (12.7)</td>
<td>&lt;0.001</td>
<td>647 (9.3)</td>
<td>727 (10.5)</td>
<td>0.170</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.0±8.5</td>
<td>55.8±8.1</td>
<td>&lt;0.001</td>
<td>55.8±9.4</td>
<td>56.5±8.5</td>
<td>0.900</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.9±5.2</td>
<td>157.4±5.5</td>
<td>&lt;0.001</td>
<td>156.7±5.4</td>
<td>156.5±5.2</td>
<td>0.900</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.9±5.5</td>
<td>56.7±5.1</td>
<td>&lt;0.001</td>
<td>65.7±6.1</td>
<td>68.1±7.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4±1.9</td>
<td>22.9±1.5</td>
<td>&lt;0.001</td>
<td>26.7±1.6</td>
<td>27.7±2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>75.3±6.2</td>
<td>80.6±5.1</td>
<td>&lt;0.001</td>
<td>87.8±6.6</td>
<td>91.2±6.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PBF (%)</td>
<td>27.0±5.3</td>
<td>30.6±4.3</td>
<td>&lt;0.001</td>
<td>35.9±4.1</td>
<td>37.4±4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM (kg)</td>
<td>15.8±1.9</td>
<td>15.8±1.9</td>
<td>0.830</td>
<td>17.1±2.1</td>
<td>17.3±2.2</td>
<td>0.052</td>
</tr>
<tr>
<td>ASM/height (kg/m²)</td>
<td>6.24±0.51</td>
<td>6.33±0.48</td>
<td>&lt;0.001</td>
<td>6.94±0.54</td>
<td>7.04±0.57</td>
<td>0.002</td>
</tr>
<tr>
<td>ASM/weight (%)</td>
<td>29.3±2.5</td>
<td>27.8±2.0</td>
<td>&lt;0.001</td>
<td>26.0±2.0</td>
<td>25.5±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASM/BMI</td>
<td>0.74±0.10</td>
<td>0.69±0.08</td>
<td>&lt;0.001</td>
<td>0.64±0.08</td>
<td>0.63±0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat measurements by CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>134.4±39.9</td>
<td>151.0±37.3</td>
<td>&lt;0.001</td>
<td>208.1±48.6</td>
<td>213.9±55.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>50.6 (32.1, 73.3)</td>
<td>94.6 (73.7, 118.9)</td>
<td>&lt;0.001</td>
<td>102.6 (81.0, 129.9)</td>
<td>139.0 (114.5, 161.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/height (cm²/m²)</td>
<td>20.1 (12.6, 29.3)</td>
<td>38.2 (30.3, 47.5)</td>
<td>&lt;0.001</td>
<td>41.9 (32.7, 53.2)</td>
<td>56.8 (46.0, 67.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/weight (cm²/kg)</td>
<td>0.93 (0.61, 1.32)</td>
<td>1.66 (1.32, 2.05)</td>
<td>&lt;0.001</td>
<td>1.57 (1.23, 2.01)</td>
<td>2.04 (1.70, 2.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFA/BMI</td>
<td>2.35 (1.57, 3.29)</td>
<td>4.14 (3.28, 5.08)</td>
<td>&lt;0.001</td>
<td>3.86 (3.08, 4.86)</td>
<td>4.95 (4.12, 5.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VSR</td>
<td>0.37 (0.27, 0.50)</td>
<td>0.63 (0.47, 0.83)</td>
<td>&lt;0.001</td>
<td>0.51 (0.37, 0.68)</td>
<td>0.63 (0.52, 0.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Muscle measurements by CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAMA (cm²)</td>
<td>109.3±12.2</td>
<td>111.6±12.1</td>
<td>&lt;0.001</td>
<td>121.9±13.5</td>
<td>126.1±14.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMA/height (cm²/m²)</td>
<td>43.3±4.7</td>
<td>45.0±4.6</td>
<td>&lt;0.001</td>
<td>49.7±5.2</td>
<td>51.4±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMA/weight (cm²/kg)</td>
<td>2.03±0.19</td>
<td>1.97±0.18</td>
<td>&lt;0.001</td>
<td>1.86±0.18</td>
<td>1.86±0.18</td>
<td>0.890</td>
</tr>
<tr>
<td>TAMA/BMI</td>
<td>5.14±0.56</td>
<td>4.89±0.050</td>
<td>&lt;0.001</td>
<td>4.56±0.48</td>
<td>4.56±0.48</td>
<td>0.870</td>
</tr>
<tr>
<td>SMA (cm²)</td>
<td>104.6±11.8</td>
<td>105.8±11.9</td>
<td>0.006</td>
<td>114.0±13.5</td>
<td>117.8±14.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA/height (cm²/m²)</td>
<td>41.5±4.5</td>
<td>42.7±4.5</td>
<td>&lt;0.001</td>
<td>46.4±5.2</td>
<td>51.4±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMA/weight (cm²/kg)</td>
<td>1.95±0.20</td>
<td>1.87±0.18</td>
<td>&lt;0.001</td>
<td>1.74±0.90</td>
<td>1.73±0.18</td>
<td>0.810</td>
</tr>
<tr>
<td>SMA/BMI</td>
<td>4.92±0.58</td>
<td>4.64±0.51</td>
<td>&lt;0.001</td>
<td>4.27±0.49</td>
<td>4.26±0.50</td>
<td>0.820</td>
</tr>
<tr>
<td>NAMA (cm²)</td>
<td>81.5±12.9</td>
<td>79.2±13.1</td>
<td>&lt;0.001</td>
<td>80.0±15.9</td>
<td>82.5±16.4</td>
<td>0.005</td>
</tr>
<tr>
<td>NAMA/height (cm²/m²)</td>
<td>32.3±4.9</td>
<td>31.9±5.1</td>
<td>0.070</td>
<td>32.6±6.3</td>
<td>33.6±6.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Variable</td>
<td>Without obesity</td>
<td>With obesity</td>
<td>P-value</td>
<td>Without obesity</td>
<td>With obesity</td>
<td>P-value</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------</td>
<td>-----------------</td>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>NAMA/weight (cm²/kg)</td>
<td>1.52±0.25</td>
<td>1.40±0.23</td>
<td>&lt;0.001</td>
<td>1.22±0.24</td>
<td>1.22±0.24</td>
<td>0.780</td>
</tr>
<tr>
<td>NAMA/BMI</td>
<td>3.84±0.69</td>
<td>3.47±0.59</td>
<td>&lt;0.001</td>
<td>3.00±0.61</td>
<td>2.99±0.63</td>
<td>0.770</td>
</tr>
<tr>
<td>IMAT (cm²)</td>
<td>3.7 (2.3, 5.8)</td>
<td>4.6 (3.2, 7.1)</td>
<td>&lt;0.001</td>
<td>6.4 (4.2, 10.3)</td>
<td>6.5 (4.3, 10.8)</td>
<td>0.460</td>
</tr>
<tr>
<td>LAMA (cm²)</td>
<td>22.1 (17.8, 27.5)</td>
<td>25.4 (21.1, 31.2)</td>
<td>&lt;0.001</td>
<td>32.9 (26.9, 39.8)</td>
<td>33.4 (28.1, 41.3)</td>
<td>0.087</td>
</tr>
<tr>
<td>NAMA/TAMA index</td>
<td>74.6±8.5</td>
<td>71.0±8.7</td>
<td>&lt;0.001</td>
<td>65.6±10.4</td>
<td>65.4±10.7</td>
<td>0.800</td>
</tr>
<tr>
<td>FLI</td>
<td>8.3±7.6</td>
<td>18.8±12.1</td>
<td>&lt;0.001</td>
<td>28.3±15.4</td>
<td>44.1±19.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FIB-4</td>
<td>-</td>
<td>1.16 (0.92, 1.44)</td>
<td>&lt;0.001</td>
<td>-</td>
<td>1.14 (0.88, 1.50)</td>
<td>0.845</td>
</tr>
<tr>
<td>FIB-4 &gt;2.67 (%)</td>
<td>-</td>
<td>0.8</td>
<td>&lt;0.001</td>
<td>-</td>
<td>2.2</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or median (interquartile range).
NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; PBF, percent body fat; ASM, appendicular skeletal muscle mass; CT, computed tomography; VFA, visceral fat area; VSR, visceral to subcutaneous fat ratio; TAMA, total abdominal muscle area; SMA, skeletal muscle area; IMAT, inter/intra-muscular adipose tissue; LAMA, low attenuation muscle area; NAMA, normal attenuation muscle area; NAMA,TAMA index=(NAMA/TAMA)×100; FLI, fatty liver index; FIB-4, fibrosis-4 index.

P-values represent the comparison between the groups with and without NAFLD by paired t-test or Mann–Whitney U-test for continuous variables and by chi-square test for categorical variables. Asterisk (*) denotes comparison vs. NAFLD without obesity group (*P<0.05; **P<0.001).
rized as mild, 31.7% as moderate, and 7.1% as severe. The number of individuals with NAFLD and the proportion of NAFLD severity according to the presence of obesity are shown in Figure 1.

**Comparison of lifestyle factors and laboratory findings according to the presence of NAFLD and obesity**

When individuals with NAFLD were compared with those without, those with NAFLD had less favorable lipid and inflammatory profiles, higher insulin resistance, and a higher prevalence of hypertension and diabetes regardless of sex (Supplementary Tables 2 and 3).

**Comparison of anthropometric and CT measurements according to the presence of NAFLD and obesity**

When individuals with NAFLD were compared with those without, those with NAFLD had higher fat measurements than those without, regardless of the presence of obesity or sex. Among non-obese individuals, the weight- and BMI-adjusted muscle measurements, NAMA (only in women), and NAMA/TAMA index were lower in those with NAFLD than in those without (Tables 1 and 2).

**Comparison of hepatic steatosis among four groups and liver fibrosis between obese and non-obese individuals with NAFLD**

The FLI was significantly higher in individuals with NAFLD than in those without, regardless of the presence of obesity or sex. Significant fibrosis (FIB-4>2.67) was higher in NAFLD with obesity than in those without obesity only in women, but median FIB-4 was not different between the two groups (Tables 1 and 2).

**Prevalence of VFO and sarcopenia or myosteatosis in individuals with NAFLD without obesity**

When non-obese individuals with NAFLD were compared with those without NAFLD, the prevalence of VFO (69.9% vs. 30.5% in total, \( P<0.001 \)), sarcopenia (31.8% vs. 21.3% in total, \( P<0.001 \)), and myosteatosis (31.7% vs. 25.3% in total, \( P<0.001 \)) were higher in those with NAFLD than in those without (Fig. 2). A prevalence analysis for each adverse body composition was performed and showed similar results in NAFLD defined by FLI≥30 (Supplementary Fig. 1). However, the prevalence of sarcopenia defined by height-adjusted SMA was lower in individuals with NAFLD than in those without (Supplementary Fig. 2).

*Figure 2. Prevalence of visceral fat obesity (VFO), sarcopenia, and myosteatosis in controls and non-alcoholic fatty liver disease (NAFLD) without obesity in men and women. Asterisk (*) denotes the comparison between controls and NAFLD without obesity (\(^*P<0.001\) by chi-squared test).*
Regression analysis according to the presence of NAFLD and obesity

To assess the roles of sarcopenia or myosteatosis and VFO on the risk of NAFLD, we performed a logistic regression analysis (Table 3). The effect of sarcopenia or myosteatosis was separately analyzed to elucidate the clinical significance of each variable. Sarcopenia or myosteatosis adjusted with age, regular exercise, VFO, diabetes, high C-reactive protein (CRP) level, and menopause (only in women) was significantly associated with NAFLD only in non-obese individuals (Table 3). However, after additional adjustment with triglyceride

Table 3. Odds ratios (OR) with 95% confidence intervals (CIs) for NAFLD according to the presence of obesity

<table>
<thead>
<tr>
<th>Model</th>
<th>Without obesity</th>
<th></th>
<th></th>
<th>With obesity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P-value</td>
<td>OR</td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>For sarcopenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>1.41</td>
<td>1.19–1.67</td>
<td>&lt;0.001</td>
<td>1.16</td>
<td>0.97–1.38</td>
<td>0.100</td>
</tr>
<tr>
<td>VFO</td>
<td>3.97</td>
<td>3.43–4.59</td>
<td>&lt;0.001</td>
<td>2.00</td>
<td>1.69–2.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.14</td>
<td>1.74–2.64</td>
<td>&lt;0.001</td>
<td>2.75</td>
<td>2.14–3.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.18</td>
<td>0.95–1.46</td>
<td>0.13</td>
<td>1.63</td>
<td>1.28–2.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>1.59</td>
<td>1.40–1.90</td>
<td>&lt;0.001</td>
<td>1.07</td>
<td>0.82–1.39</td>
<td>0.620</td>
</tr>
<tr>
<td>VFO</td>
<td>5.43</td>
<td>4.53–6.42</td>
<td>&lt;0.001</td>
<td>3.33</td>
<td>2.58–4.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.93</td>
<td>3.01–5.14</td>
<td>&lt;0.001</td>
<td>3.51</td>
<td>2.39–5.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.04</td>
<td>0.79–1.39</td>
<td>0.770</td>
<td>2.11</td>
<td>1.55–2.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Menopause</td>
<td>1.73</td>
<td>1.28–2.33</td>
<td>&lt;0.001</td>
<td>1.61</td>
<td>1.04–2.49</td>
<td>0.031</td>
</tr>
<tr>
<td>For myosteatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myosteatosis</td>
<td>1.24</td>
<td>1.02–1.50</td>
<td>0.028</td>
<td>1.06</td>
<td>0.89–1.26</td>
<td>0.500</td>
</tr>
<tr>
<td>VFO</td>
<td>3.98</td>
<td>3.44–4.60</td>
<td>&lt;0.001</td>
<td>1.99</td>
<td>1.68–2.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.18</td>
<td>1.77–2.68</td>
<td>&lt;0.001</td>
<td>2.75</td>
<td>2.14–3.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.18</td>
<td>0.95–1.46</td>
<td>0.130</td>
<td>1.64</td>
<td>1.29–2.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myosteatosis</td>
<td>1.23</td>
<td>1.04–1.46</td>
<td>0.017</td>
<td>0.92</td>
<td>0.70–1.20</td>
<td>0.540</td>
</tr>
<tr>
<td>VFO</td>
<td>5.33</td>
<td>4.51–6.31</td>
<td>&lt;0.001</td>
<td>3.30</td>
<td>2.56–4.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.97</td>
<td>3.04–5.18</td>
<td>&lt;0.001</td>
<td>3.48</td>
<td>2.37–5.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.06</td>
<td>0.80–1.41</td>
<td>0.690</td>
<td>2.13</td>
<td>1.57–2.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Menopause</td>
<td>1.70</td>
<td>1.26–2.29</td>
<td>&lt;0.001</td>
<td>1.63</td>
<td>1.05–2.91</td>
<td>0.028</td>
</tr>
<tr>
<td>For sarcopenia with myosteatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenia with myosteatosis</td>
<td>1.35</td>
<td>0.94–1.94</td>
<td>0.106</td>
<td>1.16</td>
<td>0.86–1.56</td>
<td>0.338</td>
</tr>
<tr>
<td>VFO</td>
<td>3.98</td>
<td>3.44–4.60</td>
<td>&lt;0.001</td>
<td>1.99</td>
<td>1.68–2.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.14</td>
<td>1.76–2.68</td>
<td>&lt;0.001</td>
<td>2.75</td>
<td>2.13–3.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.18</td>
<td>0.95–1.46</td>
<td>0.129</td>
<td>1.63</td>
<td>1.29–2.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopenia with myosteatosis</td>
<td>1.44</td>
<td>1.05–1.90</td>
<td>0.028</td>
<td>1.02</td>
<td>0.72–1.44</td>
<td>0.926</td>
</tr>
<tr>
<td>VFO</td>
<td>5.36</td>
<td>4.52–6.34</td>
<td>&lt;0.001</td>
<td>3.44</td>
<td>2.59–4.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.99</td>
<td>3.05–5.21</td>
<td>&lt;0.001</td>
<td>3.50</td>
<td>2.38–5.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High CRP</td>
<td>1.06</td>
<td>0.80–1.41</td>
<td>0.696</td>
<td>2.10</td>
<td>1.54–2.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Menopause</td>
<td>1.66</td>
<td>1.30–2.11</td>
<td>&lt;0.001</td>
<td>1.70</td>
<td>1.18–2.46</td>
<td>0.005</td>
</tr>
</tbody>
</table>

NAFLD, non-alcoholic fatty liver disease; VFO, visceral fat obesity; hsCRP, high sensitive C-reactive protein.
Adjusted for age, regular aerobic exercise, regular resistance exercise, VFO, diabetes, high CRP, menopausal status (only in women), and sarcopenia or myosteatosis.
(TG) level, high-density lipoprotein (HDL)-cholesterol, and hypertension, these associations remained significant only in non-obese women (Supplementary Table 4). In all subgroups divided according to sex and the presence of obesity, VFO was significantly associated with NAFLD with the highest odds ratios (Table 3). In addition, we found that sarcopenia with myosteatosis was significantly associated with an increased risk for NAFLD, especially in non-obese women (Table 3). A regression analysis of NAFLD defined by FLI is also shown in Supplementary Table 5.

**DISCUSSION**

In this study involving 14,400 individuals who underwent abdominal CT scans during routine health examinations, we found that the prevalence of NAFLD was 21.4% in non-obese individuals. This prevalence is similar to the global prevalence of NAFLD in the non-obese population (20%) but higher than those reported in previous Korean studies (7.3–18.3%). Indeed, this prevalence is much lower than the 60.4% NAFLD prevalence in individuals with obesity; however, 45.5% of NAFLD cases were found in non-obese individuals because the proportion of non-obese individuals was 70.3% of the total study population.

When we analyzed the body composition such as different fat and muscle areas by abdominal CT scan, various fat measurements such as SFA, VFA, and its adjusted indices, VSR, and IMAT were higher in both obese and non-obese individuals with NAFLD than in those without NAFLD. The NAMA (only in women) and NAMA/TAMA index, which reflect good quality muscle without myosteatosis, were lower in non-obese individuals with NAFLD than in those without. Regression analysis showed that sarcopenia and/or myosteatosis was associated with NAFLD; however, VFO was associated with a much higher risk of NAFLD in non-obese individuals. We also found that VFO, sarcopenia, and/or myosteatosis were more prevalent in non-obese individuals with NAFLD than in those without NAFLD.

Although VAT accounts for only 7% to 15% of the total body fat, it plays a more important role than other adipose depots in the pathogenesis of insulin resistance. Portal venous blood contains high levels of free fatty acids and cytokines secreted by VAT, which is thought to drive the development of NAFLD. Therefore, many studies showed that VAT is closely related to NAFLD even in non-obese individuals with NAFLD. When the subcutaneous adipose tissue (SAT), which may act as a reservoir for metabolically neutral surplus lipid storage, becomes saturated, fat deposits occur in other areas such as VAT and hepatocytes. Therefore, we used VSR in defining VFO in order to consider the different effects of VAT and SAT on NAFLD. This is consistent with a previous Korean study in which higher VSR was associated with an increased risk of NAFLD in both obese and non-obese individuals.

Sarcopenia has been shown to be associated with NAFLD and its complications such as NASH and liver fibrosis, independent of obesity. Insulin resistance can be a major pathophysiologic link between sarcopenia and NAFLD because the muscle is the primary organ responsible for insulin-mediated glucose disposal; hence, a decreased muscle mass may cause impaired glucose metabolism. Our current study also showed that the presence of sarcopenia was significantly associated with a higher risk for NAFLD. This result is similar to previous studies in which sarcopenia defined by BMI-adjusted abdominal muscle area was significantly associated with the risk of NAFLD. However, a recent study reported that there was no significant association between sarcopenia defined by height-adjusted abdominal muscle area and NASH. This is similar to the longstanding disagreements about whether higher skeletal muscle mass is associated with metabolic healthy or unhealthy phenotype, and we suggested that this inconsistency was due to differences in the adjustments for muscle mass. Determining the ideal method of adjustment for muscle mass among height, weight, and BMI has long been a matter of debate in the discussion about sarcopenia, especially in Asian populations, because adjustment with height could lead to an underestimation of sarcopenia, especially in women. Previous studies on age-related changes in muscle mass or quality of lumbar skeletal muscle area compared the prevalence of sarcopenia or myosteatosis with height-, weight-, or BMI-adjusted indices and showed that BMI-adjusted index may be a more reasonable index for diagnosing sarcopenia and myosteatosis. Furthermore, the Foundation for the National Institutes of Health Sarcopenia Project recommended using appendicular skeletal muscle (ASM)/BMI for the diagnosis of sarcopenia considering that BMI adjustment is most strongly and directly correlated with weakness and slowness based on large population-based studies.
We observed that myosteatosis, represented by a lower NAMA/TAMA index, was significantly associated with non-obese NAFLD; however, its contribution was relatively smaller than that of VFO. In our previous study, a higher NAMA/TAMA index, which is an index for good quality muscle, was negatively associated with NAFLD and fibrosis indices. This finding is consistent with a previous study, which showed that among non-obese women, myosteatosis was more common in those with NAFLD than in those without NAFLD. Other studies reported similar results in which muscle fat content, as measured by muscle attenuation, was associated with biopsy-proven NASH and fibrosis, or its progression. While height-adjusted muscle mass measured by CT scan was associated with fibrosis in one study, other studies did not find such an association.

While insulin resistance may be a major pathophysiologic link for the association between VFO, sarcopenia, or myosteatosis and NAFLD without obesity, oxidative stress occurring as the result of chronic low-grade inflammation could be another important factor. We found that hsCRP was higher in non-obese individuals with NAFLD than in those without NAFLD regardless of sex, although hsCRP was not independently associated with NAFLD without obesity in regression analysis.

For the management of non-obese individuals with NAFLD, many clinical observations suggest that weight reduction or increased physical activity may lead to improvement of hepatic steatosis and fibrosis because weight gain, even within a non-obese range, was associated with the development of NAFLD. However, our study suggests that improvement of body composition (e.g., reduction of VAT, increase of skeletal muscle mass, and improvement of myosteatosis) may be more important than simple weight reduction in managing NAFLD in non-obese individuals. Therefore, proper resistance exercise in addition to aerobic exercise or physical activity could be recommended as lifestyle modifications for non-obese individuals with NAFLD. To develop a standardized recommendation, prospective controlled studies for proper exercise protocol are needed.

Our study is limited in that the study population was composed of those who visited one health screening center for regular health examinations, which is prone to selection bias and limited generalizability. Nevertheless, a previous study from this population showed that the patterns of body composition according to age and sex were similar to the nationally representative data from the Fourth Korean National Health and Nutrition Examination Surveys. Second, the cross-sectional nature of this study did not allow us to investigate the causal relationships between these measurements and NAFLD. Third, we could only assess whether anti-diabetic medications were being taken through a questionnaire survey. In most cases, it is not possible to determine the type of medication being used. However, the proportion of patients using TZD is very low among those who can be identified. Therefore, the impact on the overall result is considered insignificant. Lastly, we diagnosed NAFLD with ultrasonographic examination instead of liver biopsy, which is the gold standard method. Therefore, we performed an additional analysis with the generally accepted surrogate markers of hepatic steatosis and fibrosis.

Nevertheless, our study has several strengths including large sample size, thorough measurements, and rigorously controlled data after the exclusion of health conditions that may affect body composition such as cancer or hyperthyroidism. In addition to measurements of VAT and SAT, measurements of skeletal muscle mass and myosteatosis could contribute to improving our understanding of the association between NAFLD and body compositional characteristics.

In conclusion, this study showed that VFO, sarcopenia, and/or myosteatosis were significantly associated with non-obese NAFLD. We also found that although VFO is the most important risk factor, both sarcopenia and myosteatosis may also be meaningful risk factors for non-obese NAFLD. These results suggest that improvement of body composition, including reducing VAT, increasing skeletal muscle mass, and improving myosteatosis, should be considered for managing NAFLD in non-obese individuals.

Authors’ contribution

HK Kim: designed the research, performed the statistical analysis, interpreted the results, wrote the manuscript, and took primary responsibility for the final content; SJ Bae: interpreted the results and wrote the manuscript; MJ Lee, EH Kim, J Choe, YK Cho, CH Jung, and WJ Lee: discussed the results and reviewed the final manuscript; HS Kim: collected data and reviewed the final manuscript; HN Park: performed critical revision; all authors read and approved the final version of the manuscript.

https://doi.org/10.3350/cmh.2023.0035
http://www.e-cmh.org
Acknowledgements
We thank Dr. Joon Seo Lim from the Scientific Publications Team at Asan Medical Center for his editorial assistance in preparing this manuscript.

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


43. Hsieh YC, Joo SK, Koo BK, Lin HC, Kim W. Muscle alterations are independently associated with significant fibrosis in patients with nonalcoholic fatty liver disease. Liver Int 2021;41:494-504.
Differences in liver and mortality outcomes of non-alcoholic fatty liver disease by race and ethnicity: A longitudinal real-world study

Vy H. Nguyen\textsuperscript{1,2,*}, Isaac Le\textsuperscript{1,3,*}, Audrey Ha\textsuperscript{1}, Richard Hieu Le\textsuperscript{1,4}, Nicholas Ajit Rouillard\textsuperscript{1}, Ashley Fong\textsuperscript{1}, Surya Gudapati\textsuperscript{1,5}, Jung Eun Park\textsuperscript{1}, Mayumi Maeda\textsuperscript{1}, Scott Barnett\textsuperscript{1}, Ramsey Cheung\textsuperscript{1,6}, and Mindie H. Nguyen\textsuperscript{1,7}

\textsuperscript{1}Division of Gastroenterology and Hepatology, Stanford University Medical Center, Palo Alto, CA; \textsuperscript{2}Harvard Medical School, Boston, MA; \textsuperscript{3}Emory University, Atlanta, GA; \textsuperscript{4}William Carey University College of Osteopathic Medicine, Hattiesburg, MS; \textsuperscript{5}Washington University, St Louis, MO; \textsuperscript{6}Division of Gastroenterology and Hepatology, Palo Alto Veterans Affairs Medical Center, Palo Alto, CA; \textsuperscript{7}Department of Epidemiology and Population Health, Stanford University Medical Center, Palo Alto, CA, USA

Graphical Abstract

Study Highlights
- What is already known in this topic?
  Data on racial and ethnic disparities in long-term liver-related events and mortality outcomes of patients with non-alcoholic fatty liver disease (NAFLD) in the United States remained sparse.
- What this study adds?
  Black and Hispanic patients had significantly higher overall mortality risk compared to White patients with NAFLD. Black patients also had up to three times greater 10-year cumulative mortality incidence compared to other racial and ethnic groups.
- How this study may affect research, practice or policy?
  Interventions that are culturally sensitive to the needs of different racial and ethnic communities are needed to address specific barriers to care to improve outcomes.
INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease in which steatosis is present in greater than 5% of the liver cells. NAFLD is a progressive disease in up to 20% of patients, but until recently, there has been a paucity of non-invasive tests for both steatosis and fibrosis diagnoses, so our understanding of progressive NAFLD has mostly come from those who have undergone a liver biopsy or abdominal imaging which limits large population studies.\(^1\)\(^-\)\(^6\)

As such, our understanding of the factors associated with adverse outcomes of NAFLD, such as race and ethnicity, is evolving. From prior studies conducted in the United States, Hispanic origin is associated with the highest risk of having NAFLD, while those of non-Hispanic Black origin have a lower risk of having NAFLD but a higher risk for adverse outcomes, including mortality.\(^17\)\(^-\)\(^23\) However, the majority of prior studies on race, ethnicity, and NAFLD used data from large population-based databases that preclude survival analysis or were limited by the availability of follow-up outcomes.

Therefore, the purpose of this study was to use individual patient-level data from a large medical center to provide a
longitudinal picture of the role of ethnicity in patients residing in the United States who have NAFLD.

MATERIALS AND METHODS

Study design and study population

We retrospectively identified patients with NAFLD at Stanford University Medical Center, Palo Alto, California, USA, between 1995 and 2021. NAFLD was confirmed by the presence of hepatic steatosis in abdominal ultrasound, computed tomography, or magnetic resonance imaging on manual chart review. We excluded patients with significant alcohol use and/or concurrent viral hepatitis, autoimmune hepatitis, alpha-1 antitrypsin deficiency, hemochromatosis, or Wilson’s disease. Data on race and ethnicity are self-reported by the patients. Patients with unknown, mixed race and ethnicity, or race and ethnicity other than White, Black, Hispanic, or Asian, were excluded due to small numbers. The final study cohort was grouped into four race and ethnicity groups: White, Black, Hispanic, and Asian. Study data were obtained via individual chart review of included patients with mortality data supplemented/confirmed by National Death Index search. The study was approved by the Institutional Review Board at Stanford University, Stanford, CA. All authors had access to the study data and reviewed and approved the final manuscript.

Study outcomes and study definitions

The primary study outcomes included the incidence of liver events and overall and non-liver-related mortality. Liver-related outcomes included the development of NAFLD-i (defined as NAFLD with stage 1 fibrosis or higher), cirrhosis, hepatocellular carcinoma (HCC), and/or liver-related deaths, whichever came first. Cirrhosis was defined by liver histology; clinical diagnosis of portal hypertension, platelet <120,000/μL, history of ascites and/or hepatic encephalopathy; by radiographic findings such as nodular liver contour; or by non-invasive methods (Fibrosure®, FIB-4 >3.25, shear wave ultrasound, Fibroscan®, or magnetic resonance elastography).

The study observation period began at the time NAFLD was confirmed, and the censor criteria included the development of study outcomes, loss to follow-up, death, or end of the study period, whichever came first.

Statistical analysis

We described and compared continuous variables among the 4 study groups using the analysis of variance test if the variables followed a normal distribution and the Kruskal–Wallis test if not. We reported results for continuous variables as mean (±standard deviation) or median and interquartile range. For categorical variables, we reported data as numbers and percentages (%) and used the χ² test to compare values among groups.

We used the Kaplan–Meier methods to determine the incidence of liver-related outcomes, overall mortality, and non-liver-related mortality. We used the log-rank test to compare the incidence of events of interest among the study groups.

We used univariable Cox proportional hazards regression to estimate the unadjusted hazard ratio (HR) and identify potential factors (with P<0.10) to include in the multivariable model to estimate adjusted hazard ratios (aHR) for factors associated with the development of liver events, overall or non-liver related mortality. Factors with potential association with outcomes by prior reports were also included in the multivariable models. Statistical significance was defined with a two-tailed P-value <0.05, and all analyses were done using the Stata version 17 (Stata Corporation, College Station, TX, USA).

RESULTS

Patient characteristics

Our study cohort included a total of 9,340 NAFLD patients who met our study criteria. The study patients were divided into four groups: White (4,115 patients, 44.1%), Black (214 patients, 2.3%), Hispanic (2,604 patients, 27.9%), and Asian (2,407 patients, 25.7%) (Table 1). Hispanic patients were the youngest group with a mean age of 44.5 years, about 10 years younger than the White patients (mean age 54.1 years), followed by Asian and Black patients (mean age 48.3 and 51.4 years, respectively) (P<0.0001). The Hispanic group was most likely to be female (63.3%) while the majority of patients in the Asian group were males (55.5%) (P<0.0001).
Black patients had the highest body mass index (BMI) and the highest percentage of diabetes mellitus, hypertension, cardiovascular disease, and chronic kidney disease. In fact, the majority of Black patients in the cohort had hypertension (65.9%), close to one-half had diabetes mellitus (42.1%), and about one in three (30.4%) had chronic kidney disease. Patients in the Black group had the lowest aspartate aminotransferase, alanine aminotransferase, and the highest platelet levels compared to other groups, but they had the highest alkaline phosphatase (Table 2). However, we found no significant difference in the total cholesterol, triglycerides, or glucose levels among the four racial and ethnic groups. White patients were most likely to have non-liver cancer (23.0% compared to 13.1–14.8% in other groups, \( P < 0.0001 \)). The percentage of cirrhosis was lowest in the Asian group (15.5%), followed by the Hispanic group (17.8%), and highest in the White (21.8%) and Black (23.3%) groups \( (P < 0.0001) \) (Table 1). Notably, while Black patients made up 2.29% of the total cohort, only 0.67% of the liver biopsies were performed in Black patients as compared to 49.11% among White patients who made up 44.06% of the cohort.

Table 1. Baseline characteristics of patients with non-alcoholic fatty liver disease by race and ethnicity

<table>
<thead>
<tr>
<th>Variable</th>
<th>White (n=4,115)</th>
<th>Black (n=214)</th>
<th>Hispanic (n=2,604)</th>
<th>Asian (n=2,407)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>54.1±14.9</td>
<td>51.4±15.2</td>
<td>44.5±15.2</td>
<td>48.3±15.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49.2</td>
<td>61.2</td>
<td>63.3</td>
<td>44.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male</td>
<td>50.8</td>
<td>38.8</td>
<td>37.7</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>32.3±6.7</td>
<td>36.1±9.9</td>
<td>33.5±7.5</td>
<td>28.0±5.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27.2</td>
<td>42.1</td>
<td>29.8</td>
<td>29.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>47.5</td>
<td>65.9</td>
<td>34.0</td>
<td>29.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>47.4</td>
<td>45.8</td>
<td>32.1</td>
<td>52.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>9.5</td>
<td>14.0</td>
<td>6.7</td>
<td>5.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>25.6</td>
<td>30.4</td>
<td>21.7</td>
<td>19.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-liver cancer</td>
<td>23.0</td>
<td>13.1</td>
<td>14.8</td>
<td>13.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIB-4 index</td>
<td>1.4±0.6</td>
<td>1.4±0.6</td>
<td>1.3±0.5</td>
<td>1.3±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>21.8</td>
<td>23.3</td>
<td>17.8</td>
<td>15.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or %. FIB-4, fibrosis-4 index.

Table 2. Baseline laboratory characteristics of patients with non-alcoholic fatty liver disease by race and ethnicity

<table>
<thead>
<tr>
<th>Variable</th>
<th>White (n=4,115)</th>
<th>Black (n=214)</th>
<th>Hispanic (n=2,604)</th>
<th>Asian (n=2,407)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>44 (28–71)</td>
<td>35.5 (24–63)</td>
<td>46 (30–79)</td>
<td>46 (30–75)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AST</td>
<td>30 (22–45)</td>
<td>25 (19–46)</td>
<td>31 (22–51)</td>
<td>30 (22–43)</td>
<td>0.041</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>94.9±73.6</td>
<td>103.9±114.5</td>
<td>102.6±59.1</td>
<td>87.5±53.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.98±1.4</td>
<td>1.0±0.7</td>
<td>0.8±0.5</td>
<td>0.9±0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelets</td>
<td>246.6±84.3</td>
<td>266.2±81.3</td>
<td>259.4±80.6</td>
<td>254.0±77.8</td>
<td>0.0079</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>188.9±46.8</td>
<td>185.5±45.7</td>
<td>186.4±52.0</td>
<td>190.3±43.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>169.2±263.4</td>
<td>143.9±116.3</td>
<td>194.9±241.0</td>
<td>180.5±155.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Glucose</td>
<td>119.9±48.0</td>
<td>129.9±56.3</td>
<td>130.2±69.7</td>
<td>119.8±44.1</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Values are expressed as mean±standard deviation or median (interquartile range).

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Liver-related outcomes (NAFLD-i, cirrhosis, HCC, and liver-related mortality)

Over a follow-up of 140,167 persons-years for White patients, 5,985 persons-years for Black patients, 76,684 persons-years for Hispanic, and 97,556 persons-years for Asian patients, there were 2,711 liver-related events among White patients, 149 among Black patients, 1,898 among Hispanic patients, and 97,556 for Asian patients. Figure 1 shows that the rate of development of liver-related events differs significantly among the racial and ethnic groups (P=0.0002). The highest cumulative 5-year incidence was observed among White patients (19.1%), the lowest among Black patients (7.9%), and the Asian and Hispanic patients having fairly similar rates (14.6% and 14.5%, respectively). The difference among the White, Hispanic, and Asian groups remained significant even after Black patients, as the group with the lowest rate was excluded (P<0.0001).

On univariable Cox proportional hazard regression, compared to the White group, Black, Hispanic, and Asian groups were all associated with lower risk of NAFLD-i (aHR 0.60 to 0.82), but the association between Black patients and NAFLD-i was not statistically significant (P=0.07) (Table 3). On the multivariable model adjusted for age, sex, race and ethnicity, and diabetes mellitus, compared to White patients, only Asian patients were significantly associated with about 20% lower risk of NAFLD-i (aHR 0.81, 95% confidence interval [CI] 0.70–0.95, P=0.008) and cirrhosis (aHR 0.81, 95% CI 0.68–0.96, P=0.02). In addition, Hispanic patients had nearly four times greater risk of having liver-related mortality compared to White patients (aHR 3.84, 95% CI 1.63–9.04, P=0.002) after adjusting for age, sex, and diabetes mellitus. However, in another multivariable model adjusting for additional comorbidities such as cardiovascular diseases, chronic kidney disease, high BMI, and hyperlipidemia, we found that Black patients were less likely to have NAFLD-i and cirrhosis compared to White patients in this study (Table 3).

Overall and non-liver-related mortality

Among all death events in this study, two most common causes were cardiovascular-related (32.47%) and non-liver cancer-related (36.16%). In contrast to the findings of liver-related outcomes above, we found much higher overall and non-liver-related mortality rates among Black patients as compared to the other three groups (Fig. 2, P=0.0017 and 0.0004, respectively). Over a follow-up of in persons-years of 205,137 for White, 8,054 for Black, 103,652 for Hispanic, and 122,929 for Asian patients, there were 137, 10, 65, and 49 deaths of any cause, respectively. The 5-year and 10-year cumulative overall mortality was highest for Black patients (9.2% and 15.0%, respectively), about 3 times higher than those of the other groups (3.5% and 7.3% for White, 2.6% and 5.6% for Hispanic, and 2.5% and 4.3% for Asian groups). In a sensitivity analysis excluding Black patients as the group with the highest rate, there remained significant differences in

![Figure 1. Cumulative incidence of liver-related events (NAFLD-i, cirrhosis, liver cancer, and/or liver-related death).](https://doi.org/10.3350/cmh.2023.0205)
### Table 3. Predictors of NAFLD-i, cirrhosis, hepatocellular carcinoma, and liver-related mortality by race and ethnicity

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Number of events</th>
<th>Univariable HR (95% CI)</th>
<th>P-value</th>
<th>Multivariable HR Model 1 (95% CI)</th>
<th>P-value</th>
<th>Multivariable HR Model 2 (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NAFLD-i</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>504</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>0.54 (0.31–0.94)</td>
<td>0.03</td>
</tr>
<tr>
<td>Black</td>
<td>13</td>
<td>0.60 (0.34–1.04)</td>
<td>0.07</td>
<td>0.58 (0.34–1.01)</td>
<td>0.05</td>
<td>0.54 (0.31–0.94)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hispanic</td>
<td>232</td>
<td>0.82 (0.70–0.96)</td>
<td>0.01</td>
<td>0.99 (0.84–1.16)</td>
<td>0.89</td>
<td>1.02 (0.87–1.20)</td>
<td>0.80</td>
</tr>
<tr>
<td>Asian</td>
<td>245</td>
<td>0.72 (0.62–0.84)</td>
<td>&lt;0.0001</td>
<td>0.81 (0.70–0.95)</td>
<td>0.008</td>
<td>0.84 (0.71–0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Cirrhosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>412</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>0.56 (0.31–1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Black</td>
<td>11</td>
<td>0.63 (0.35–1.15)</td>
<td>0.13</td>
<td>0.61 (0.34–1.11)</td>
<td>0.11</td>
<td>0.56 (0.31–1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>Hispanic</td>
<td>202</td>
<td>0.89 (0.75–1.05)</td>
<td>0.17</td>
<td>1.06 (0.89–1.26)</td>
<td>0.51</td>
<td>1.10 (0.92–1.32)</td>
<td>0.29</td>
</tr>
<tr>
<td>Asian</td>
<td>199</td>
<td>0.72 (0.61–0.85)</td>
<td>&lt;0.0001</td>
<td>0.81 (0.68–0.96)</td>
<td>0.02</td>
<td>0.85 (0.71–1.03)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Hepatocellular carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>3.74 (0.71–19.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
<td>1.88 (0.38–9.30)</td>
<td>0.44</td>
<td>3.38 (0.65–17.55)</td>
<td>0.15</td>
<td>3.74 (0.71–19.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>1.15 (0.19–6.89)</td>
<td>0.88</td>
<td>1.48 (0.25–8.92)</td>
<td>0.67</td>
<td>1.94 (0.30–12.43)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Liver-related mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>3.89 (1.51–10.03)</td>
<td>0.005</td>
</tr>
<tr>
<td>Hispanic</td>
<td>13</td>
<td>2.64 (1.16–6.03)</td>
<td>0.02</td>
<td>3.84 (1.63–9.04)</td>
<td>0.002</td>
<td>3.89 (1.51–10.03)</td>
<td>0.005</td>
</tr>
<tr>
<td>Asian</td>
<td>4</td>
<td>0.65 (0.21–2.09)</td>
<td>0.47</td>
<td>0.78 (0.25–2.51)</td>
<td>0.68</td>
<td>0.88 (0.25–3.07)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

HR, hazard ratios; CI, confidence interval; NAFLD, non-alcoholic fatty liver disease; NAFLD-i, non-alcoholic fatty liver disease with stage 1 fibrosis or higher.

*a* Model 1: Adjusted for age, sex, race and ethnicity, diabetes mellitus; Model 2: adjusted for age, sex, race and ethnicity, diabetes mellitus, cardiovascular diseases, chronic kidney disease, body mass index, and hyperlipidemia. *b* Black patient group was not included due to the small sample size.
both the overall ($P=0.031$) and non-liver related ($P=0.026$) mortality among the White, Hispanic, and Asian groups.

We found that the majority of deaths in all racial and ethnic groups to be non-liver related and similar patterns of differences were among the study groups with non-liver related mortality. The numbers of non-liver-related deaths were 127, 10, 52, and 45 for White, Black, Hispanic, and Asian groups, respectively (corresponding follow-up time in persons-years: 205,137; 8,054; 103,653; and 122,929, respectively). The 5-year and 10-year cumulative rates for non-liver mortality were highest at 9.2% and 15.0% for Black, followed by White (3.2% and 6.5%), and lowest for Hispanic (2.0% and 4.0%) and Asian (2.3% and 3.9%) patients.

On multivariable regression analysis (Table 4), compared to White patients, Black patients were at more than two times higher risk of both non-liver related (aHR 2.35, 95% CI 1.22–4.51, $P=0.010$) as well as overall mortality (aHR 2.13, 95% CI 1.11–4.08, $P=0.022$). Hispanic patients also had about 50% higher risk of overall mortality compared to White patients (aHR 1.44, 95% CI 1.05–1.99, $P=0.022$), but there was no statistically significant difference between Hispanic and White patients in regard to non-liver-related mortality risk (aHR 1.23, 95% CI 0.87–1.75, $P=0.22$). Meanwhile, though not statistically significant, there was a trend for also lower risk of

**Figure 2.** Cumulative incidence of (A) overall mortality and (B) non-liver related mortality among patients with NAFLD by race and ethnicity. NAFLD, non-alcoholic fatty liver disease.
DISCUSSION

Using clinically based individual longitudinal data, we were able to closely examine the association between race and ethnicity, and long-term outcomes among patients with NAFLD in this study. We found that although Black patients had the highest cumulative incidence of cirrhosis, we removed them from our sensitivity analysis to determine the impact of race and ethnicity among White, Hispanic, and Asian patients. Because Black patients were the smallest group and carried a higher risk for overall and non-liver-related mortality compared to White patients, Asian patients were at a lower risk compared to Black patients. Asian patients also were at the highest risk for overall and non-liver-related mortality despite having a lower incidence of liver-related morbidity.

Overall, adjusted hazard ratios (HR) for overall and non-liver-related mortality among patients with NAFLD as compared to White patients in a sensitivity analysis adjusting for other comorbidities such as BMI, diabetes mellitus, cardiovascular diseases, chronic kidney disease, body mass index, and hyperlipidemia, we found similar trends and direction to the results described above except the hazard ratios for overall mortality were no longer reached statistical significance (Table 4).

Table 4. Predictors of overall mortality and non-liver related mortality among patients with NAFLD by race and ethnicity

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Number of events</th>
<th>Univariable HR (95% CI)</th>
<th>P-value</th>
<th>Multivariable HR Model 1 a (95% CI)</th>
<th>P-value</th>
<th>Multivariable HR Model 2 a (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>137</td>
<td>1</td>
<td>2.05 (1.07–3.91)</td>
<td>0.029</td>
<td>2.13 (1.11–4.08)</td>
<td>0.022</td>
<td>1.93 (0.96–3.85)</td>
</tr>
<tr>
<td>Black</td>
<td>10</td>
<td>2.05 (1.07–3.91)</td>
<td>0.029</td>
<td>2.13 (1.11–4.08)</td>
<td>0.022</td>
<td>1.93 (0.96–3.85)</td>
<td>0.06</td>
</tr>
<tr>
<td>Hispanic</td>
<td>65</td>
<td>0.95 (0.71–1.30)</td>
<td>0.77</td>
<td>1.44 (1.05–1.99)</td>
<td>0.022</td>
<td>1.53 (1.09–2.16)</td>
<td>0.02</td>
</tr>
<tr>
<td>Asian</td>
<td>49</td>
<td>0.57 (0.40–0.80)</td>
<td>0.002</td>
<td>0.70 (0.50–1.00)</td>
<td>0.053</td>
<td>0.81 (0.55–1.20)</td>
<td>0.29</td>
</tr>
<tr>
<td>Non-liver-related mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>127</td>
<td>1</td>
<td>2.22 (1.16–4.25)</td>
<td>0.015</td>
<td>2.35 (1.22–4.51)</td>
<td>0.010</td>
<td>2.05 (1.02–4.12)</td>
</tr>
<tr>
<td>Black</td>
<td>10</td>
<td>2.22 (1.16–4.25)</td>
<td>0.015</td>
<td>2.35 (1.22–4.51)</td>
<td>0.010</td>
<td>2.05 (1.02–4.12)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hispanic</td>
<td>52</td>
<td>0.81 (0.57–1.13)</td>
<td>0.227</td>
<td>1.23 (0.87–1.75)</td>
<td>0.22</td>
<td>1.33 (0.91–1.93)</td>
<td>0.14</td>
</tr>
<tr>
<td>Asian</td>
<td>45</td>
<td>0.56 (0.39–0.81)</td>
<td>0.002</td>
<td>0.70 (0.48–1.01)</td>
<td>0.059</td>
<td>0.81 (0.53–1.22)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

NAFLD, non-alcoholic fatty liver disease; HR, hazard ratios; CI, confidence interval.

a Model 1: Adjusted for age, sex, race and ethnicity, diabetes mellitus; Model 2: adjusted for age, sex, race and ethnicity, diabetes mellitus, cardiovascular diseases, chronic kidney disease, body mass index, and hyperlipidemia.

On the other hand, these data provide additional information on long-term outcomes among persons with NAFLD in the United States, an area that has been under-reported due to the use of cross-sectional data. We found that Black patients comprise a smaller proportion among those with NAFLD compared to their overall incidence of overall and non-liver-related mortality. Future studies should consider these differences when planning targeted interventions.
with NAFLD carry a substantial risk for overall mortality and non-liver-related mortality outcomes, followed by White, Hispanic, and Asian patients. These findings hold true after adjusting for the clinical differences between the groups. These results hold significance for policymakers as although Black individuals may have a lower susceptibility to developing NAFLD with fibrosis, but once present, they are disproportionately affected. Therefore, continued actions are needed to prevent the development and progression of NAFLD in Black patients and address barriers to healthcare. Hispanic patients also appear to be affected by various social determinants of health that increase their risk of developing NAFLD, so efforts in determining culturally sensitive and appropriate healthy living interventions are needed in these communities.

These recommendations take on more significance for Hispanic and Black females as they not only comprised the largest group among Black and Hispanic individuals but results from a recent study found that Hispanic and Black females experienced significant increases in the liver transplant waitlist due to non-alcoholic steatohepatitis (NASH). In fact, this study reported that NASH was the second leading indication for liver transplantation overall but the number one indication among women, especially in Hispanic and Black females. In addition, a previous study highlighted that Black patients who developed HCC after 2010 had worse survival compared to White patients due to their more advanced stage at presentation, while race and ethnicity was not an independent predictor for mortality, highlighting again the need to improve access to healthcare for Black patients. Most importantly, Black patients were significantly more likely to have comorbidities such as higher BMI, hypertension, diabetes mellitus, hyperlipidemia, and cardiovascular and chronic kidney diseases, which are all well-documented risk factors for worse health outcomes and mortality in this group. In fact, 60% of the deaths among Black patients in our study were due to cardiovascular diseases. The causes of these disparities are multifactorial and likely due to social and structural determinants of health, such as structural racism and income inequality, that together limit access to care and early diagnosis, education, and intervention.

On the other hand, Asian patients were at lower risk for NAFLD-i and cirrhosis compared to White patients and marginally at lower risk for overall and non-liver-related mortality compared to White patients. Such findings are in line with what has been reported in prior studies. In one specific study conducted among patients with HCC, investigators determined that Asian patients had improved survival compared to White patients. The investigators suggested that their improved survival could be due to genetic differences that altered the detrimental effects of factors associated with severe disease development. Although further research is needed to understand this premise among patients with NAFLD, this reasoning may be plausible as Asian patients in our study also had the lowest prevalence of cirrhosis.

Though our study was conducted retrospectively at a single tertiary care center, the cohort was large and racially and ethnically diverse, with a large proportion of Hispanic and Asian patients, and spanned over 25 years. Patients were followed longitudinally, and the study data reflected a collective experience of more than 400,000 person-years. We minimized the risk of selection bias by selecting the cohort consecutively and included patients from all clinics and diverse clinical settings in our healthcare system and not just gastroenterology or liver clinics. With the recent announcement of the metabolic dysfunction-associated steatotic liver disease (MASLD) nomenclature, which highlights the cardio-metabolic factors affecting steatotic liver disease, our studies found consistent results with Black patients who had more metabolic risks at presentations had the highest mortality risk compared to patients in other racial and ethnic groups. This further highlights the multifactorial disease pathophysiology of fatty liver disease and metabolic factors as major contributors to worse outcomes. Even though we used the NAFLD definition in our study, these results are still likely applicable with the new MASLD classification, as a recent study has shown that the discrepancy between NAFLD and MASLD is minimal, and findings from NAFLD studies should still be valid even with the nomenclature change.

In this large cohort of patients with NAFLD who were followed longitudinally in a major medical center in Northern California, we were able to determine long-term outcomes, including mortality by race and ethnicity. Although Black patients comprised the smallest proportion of our study cohort, they had the worst mortality outcomes. Black patients were at more than 2 times higher risk for both overall and non-liver-related mortality compared to White patients. Hispanic patients were 1.5 times increased risk for overall mortality compared to White patients, while Asian patients were 19% less likely to develop NAFLD-i and cirrhosis. As our under-
standing of NAFLD pathophysiology is expanded, our findings extend previous reports that used cross-sectional data and provide further evidence that policymakers need to develop interventions that are culturally appropriate and sensitive to the needs of different communities to help improve success.

**Authors’ contribution**

Study design: Vy H. Nguyen, Mindie H. Nguyen. Data analysis: Isaac Le, Vy H. Nguyen, Scott Barnet, Mindie H. Nguyen. Data collection: All authors. Drafting of manuscript: Vy H. Nguyen, Isaac Le, Mindie H. Nguyen. Data interpretation, review and revision of manuscript: all authors.

**Conflicts of Interest**

Mindie H. Nguyen: Research funding: Pfizer, Enanta, Gilead, CurveBio, Exact Sciences, Hello Health, Glycotest, National Cancer Institute, B.K. Kee Foundation, Vir Biotech; Consulting: Gilead, Intercept, GSK, Exact Science, Novartis, Janssen, Bayer.

Ramsey Cheung: Research funding: Gilead, Siemens Healthineers.

**REFERENCES**

18. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, et al. Preva-
Loco-regional therapies competing with radiofrequency ablation in potential indications for hepatocellular carcinoma: A network meta-analysis

Ha Il Kim, Ji Hyun An, Seungbong Han, and Ju Hyun Shim

1Gastroenterology and Hepatology, Hanyang University College of Medicine, Guri; 2Biostatistics, College of Medicine, Korea University, Seoul; 3Asan Liver Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul; 4Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Study Highlights
• In a network meta-analysis of 19 randomized trials exploring 11 loco-regional therapies, only the combination of TACE and RFA showed a significant improvement in the overall survival of patients with HCC measuring ≤5 cm, compared to RFA alone (HR, 0.52; 95% CI, 0.33–0.82). This combination treatment also ranked first based on P-score (0.964).
• An analysis of overall progression-free survival involving eight treatments in ten trials revealed that the HR for the combination therapy was significantly better than that for RFA alone (HR, 0.58; 95% CI, 0.38–0.89), again having the highest P-score (0.999).
• No modalities outperformed RFA alone in terms of local progression-free survival rates.
INTRODUCTION

Surgical resection and liver transplantation are the mainstays of curative treatment for early hepatocellular carcinoma (HCC) within the Milan criteria for tumor size and number.\textsuperscript{1,2} Radiofrequency ablation (RFA) has achieved comparable outcomes to surgery, with fewer complications, in several randomized controlled trials (RCTs) and remains the standard of care for patients with small HCCs that are unresectable or medically inoperable, or for whom a donor is not available.\textsuperscript{3,4} However, despite advances in imaging guidance and efforts to improve procedural safety,\textsuperscript{5} poor accessibility to intrahepatic tumors, especially in the setting of cirrhosis, may limit the adoption of RFA. \textsuperscript{5}

Background/Aims: There is no clear consensus on the relative ranking of interventional and radiation techniques with indications similar to those of radiofrequency ablation (RFA) for the treatment of early hepatocellular carcinoma (HCC). We used a network meta-analysis to compare the efficacy of non-surgical treatments for early HCC.

Methods: We searched databases for randomized trials assessing the efficacy of loco-regional treatments for HCCs ≤5 cm with no extrahepatic spread or portal invasion. The primary outcome was the pooled hazard ratio (HR) for overall survival (OS), and secondary outcomes included overall and local progression-free survival (PFS). A frequentist network meta-analysis was performed, and the relative ranking of therapies was assessed with P-scores.

Results: Nineteen studies comparing 11 different strategies in 2,793 patients were included. Chemoembolization plus RFA improved OS better than RFA alone (HR 0.52, 95% confidence interval [CI] 0.33–0.82; P-score=0.951). Cryoablation, microwave ablation, laser ablation, and proton beam therapy had similar effects on OS compared with RFA. For overall PFS, but not local PFS, only chemoembolization plus RFA performed significantly better than RFA (HR 0.61, 95% CI 0.42–0.88; P-score=0.964). Injection of percutaneous ethanol or acetic acid was significantly less effective than RFA for all measured outcomes, while no differences in progression outcomes were identified for other therapies included in the network.

Conclusions: Our results suggest that chemoembolization combined with RFA is the best option for local treatment of early HCC. Cases with potential contraindications for RFA may benefit from a tailored approach using thermal or radiation modalities. (Clin Mol Hepatol 2023;29:1013-1028)

Keywords: Hepatocellular carcinoma; Loco-regional treatment; Network meta-analysis; Survival; Radiofrequency ablation

Corresponding author: Ju Hyun Shim
Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, 88, Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-3190, Fax: +82-2-485-5782, E-mail: s5854@amc.seoul.kr
https://orcid.org/0000-0002-7336-1371

Seungbong Han
Department of Biostatistics, College of Medicine, Korea University, 73, Goryeodae-ro, Seongbuk-gu, Seoul 02841, Korea
Tel: +82-2-2286-1425, Fax: +82-2-2286-1438, E-mail: hanseungbong@gmail.com
https://orcid.org/0000-0003-2938-8072

*Hi Kim and J An contributed equally to this work.

Editor: Ju Dong Yang, Cedars-Sinai Medical Center, USA

Received: Mar. 29, 2023 / Revised: Jul. 3, 2023 / Accepted: Jul. 4, 2023

Abbreviations:
CA, cryoablation; CI, confidence interval; CTP, Child-Turcotte-Pugh; HCC, hepatocellular carcinoma; HR, hazard ratio; LA, laser ablation; MWA, microwave ablation; NMA, network meta-analysis; OS, overall survival; PEI, percutaneous ethanol injection; PBT, proton beam therapy; PEL, percutaneous ethanol injection; PFS, progression-free survival; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; RFA, radiofrequency ablation; SBRT, stereotactic body radiation therapy; SE, standard error; TACE, transarterial chemoembolization; TTP, time-to-progression

hepatic lesions as well as proximity to blood vessels or biliary ducts limit the broad application of RFA.\textsuperscript{6,7} Since the initial clinical implementation of RFA, prospective and retrospective studies on other interventional and radiation techniques have reported favorable outcomes in terms of local tumor progression and life expectancy. Several milestone trials of loco-regional methods in early HCC have focused on alternative primary strategies such as microwave ablation (MWA), cryoablation (CA), stereotactic body radiation therapy (SBRT), and proton beam therapy (PBT). These approaches, alone or combined with transarterial chemoembolization (TACE), are considered the next standard option for tumors within the Milan criteria according to international guidelines.\textsuperscript{1,8-12}

There is no consensus on the relative effectiveness of these local treatments because there is a lack of high-level direct evidence from head-to-head pairwise comparisons. Moreover, previous meta-analyses did not consider all relevant interventions, including radiotherapies, that had comparable efficacies, as demonstrated in randomized or matched-pair studies, and that were useful alternatives to standard therapy in HCC indicated for RFA.\textsuperscript{13,14} Before the results of pairwise treatments become available, there is an urgent need for evidence-based indicators of the most appropriate procedure for early unresectable tumors. To address this critical real-life question, we conducted a systemic literature review of prospective pairwise comparisons of different types of loco-regional HCC treatments. Based on our findings, we performed a network meta-analysis (NMA) comparing treatment efficacy in terms of controlling target lesions and improving patient survival and then estimated the relative benefits of individual approaches by combining direct and indirect evidence.

**MATERIALS AND METHODS**

This study adhered to the standard guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension statement for network meta-analysis (PRISMA-NMA),\textsuperscript{15} and the study was registered in PROSPERO (protocol no. CRD42021278742). The institutional review board of Asan Medical Center approved this trial-level NMA, and the requirement for informed consent of individual patients was waived (IRB No. 2021-0823). Data generated or analyzed during the study are available from the corresponding authors by request.

**Eligibility criteria**

RCTs published as full-text articles in peer-reviewed journals that prospectively examined the efficacy of loco-regional therapies for primary or recurrent HCCs ≤5 cm without extrahepatic spread or portal invasion, which are generally accepted as standard indications for RFA, were eligible for this NMA.\textsuperscript{11,16} We included studies comparing single or combined modalities with alternative intervention(s) that reported extractable data for at least one measure of overall survival (OS) and local or overall recurrence/progression. Only full text articles published in English in peer-reviewed journals were included.

Studies that had any arm of liver resection or transplantation or those that included any HCC >5 cm in diameter, which can be difficult to ablate, were excluded from the analysis. Duplicates, letters, conference proceedings, meeting abstracts, and prospective or retrospective studies of non-randomized design were also excluded.

**Search strategy and selection criteria**

Two separate literature searches were conducted to identify studies relevant to this NMA. The primary search was conducted on March 17, 2021, using the following databases: PubMed, EMBASE, Cochrane Library, CINAHL, and Web of Science. The search was limited to articles published in English between January 1, 2000, and March 17, 2021. The updated search was conducted on February 17, 2023, and used the same search strategy and databases to identify any newly published studies that met the inclusion criteria. Furthermore, we checked references cited in the relevant systematic reviews and meta-analyses. The detailed search strategy with query terms is presented in Supplementary Table 1. Two reviewers (JA and HIK, with 12 and 6 years of experience in the field of HCC treatment, respectively) independently screened all titles and abstracts identified by the searches and then scrutinized all the full manuscripts containing potentially relevant studies selected by either reviewer using the predefined criteria. Any discrepancies were resolved by discussion and consensus, or with the participation of an additional reviewer (JHS, with 19 years of experience in the field of HCC treatment).
Outcome definition and data extraction

The primary outcome was OS defined as the time from the date of enrollment to death from any cause. Secondary outcomes were overall progression-free survival (PFS) and local PFS. Based on the definition of endpoints used in all the included trials, overall progression included the following local and distant tumor events: (1) local progression defined as intrahepatic tumor recurrence or progression onto or along the peripheral margin of the treated lesion; and (2) development of any new HCC remote from the treated site, in any location, defined as distant progression. Overall PFS was measured by the interval from enrollment to either local/distant progression or death, whichever was first. For studies in which PFS was not reported as an endpoint, time elapsed to objective tumor progression or recurrence (TTP) was substituted as the secondary outcome in the NMA. The principal data extracted or derived from the included studies were the log hazard ratio (HR) and standard error (SE) or relevant information allowing estimation of HR and SE (e.g., an HR and confidence interval [CI] or P-value for survival, recurrence, and/or progression). All data were publicly available or computable from the individual studies. Additional summary data including details of study design, treatment methods, and numbers of patients and their demographics were also extracted (Table 1).

Risk of bias assessment

Risk of bias was assessed for each study independently by two of the authors (JA and HIK) using the revised Cochrane risk-of-bias tool for RCTs. 

Statistical analysis

To conduct the NMA, we required the log HRs for the survival outcomes and the corresponding SEs. However, for studies reporting only the P-value for the log-rank test along with the total number of events (e.g., deaths or progressions) for comparison groups, we derived the log HRs and SEs indirectly from the log-rank test results. For studies with no available log-rank test or log HRs, we calculated the log HRs and SEs indirectly from the Kaplan–Meier curves after constructing >15 time intervals. After obtaining the summary statistics for the survival outcomes, we derived the therapeutic hierarchy for several therapies from an NMA in which we treated RFA as the control group.

We initially fitted both the fixed effects and random effects models simultaneously and evaluated study heterogeneity across the included trials. Statistical heterogeneity was calculated using the Higgins and Thomson $I^2$ statistic, which describes the percentage of total variation across studies due to heterogeneity rather than chance. We defined the substantial heterogeneity range as comprising values $>50\%$. We also considered Cochran’s Q test for heterogeneity. Since there was no substantial evidence for heterogeneity across the studies with respect to any endpoint, we reported the results for the fixed effect model only. For direct and indirect comparisons between interventional and radiation techniques, we used a frequentist NMA approach employing weighted least squares regression. In order to rank the treatments in terms of superiority, we computed the P-score for each treatment, which measures the degree of certainty that the treatment is better than another treatment, averaged over all the competing treatments. We also performed subgroup analyses for the sub-networks involving studies based on HCC nodules ≤ 3 cm. Details of the statistical methods are provided in the Supplementary methods. All data analyses were performed using R packages (version 4.0.4.) netmeta and gemtc to conduct the main NMA and network-meta regression.

RESULTS

Study selection and characteristics

Figure 1 shows a flowchart of the study selection process. A total of 4,209 titles and abstracts of potentially relevant studies were screened. Of these, 48 fulfilled the eligibility criteria for full-text assessment. We examined the references in the relevant systematic reviews but identified no new records, as all references were already included in our database search results. Of the 48 studies, 29 were excluded after applying the exclusion criteria (Supplementary Table 2). Finally, 19 RCTs investigating 11 interventions in 2,793 patients were used for the NMA; these included the following treatment arms: RFA (1,124 patients in 15 trials), TACE+RFA (115 in 2), MWA (276 in 4), CA (180 in 1), laser ablation (LA; 70 in 1), PBT (72 in 1), TACE (84 in 1), TACE+MWA (89 in 1), per-
<table>
<thead>
<tr>
<th>Study name (Country)</th>
<th>Arm</th>
<th>No. of patients</th>
<th>Inclusion criteria for tumor</th>
<th>Main tumor size* (cm)</th>
<th>Single HCC (%)</th>
<th>HCC ≤3 cm (%)</th>
<th>Male sex (%)</th>
<th>Age* (years)</th>
<th>Follow-up duration † (months)</th>
<th>CTP class A/B/C (%)</th>
<th>Outcome</th>
<th>Major complications ‡ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdelaziz et al. 2014 (Egypt)</td>
<td>RFA</td>
<td>45</td>
<td>All &lt;5 cm, number ≤3</td>
<td>2.95</td>
<td>86.7</td>
<td>61.5</td>
<td>68.9</td>
<td>56.8</td>
<td>27</td>
<td>53.3/46.7/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>MWA</td>
<td>66</td>
<td></td>
<td>2.90</td>
<td>86.4</td>
<td>72.4</td>
<td>72.7</td>
<td>53.6</td>
<td>27</td>
<td>37.9/62.1/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td>Brunello et al. 2008 (Italy)</td>
<td>PEI</td>
<td>69</td>
<td>All ≤3 cm, number ≤3</td>
<td>2.25</td>
<td>78.7</td>
<td>100</td>
<td>71.0</td>
<td>70.3</td>
<td>26</td>
<td>56.5/43.5/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>70</td>
<td></td>
<td>2.42</td>
<td>77.1</td>
<td>100</td>
<td>61.4</td>
<td>69</td>
<td>25</td>
<td>54.3/45.7/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td>Chong et al. 2020 (Hong Kong)</td>
<td>MWA</td>
<td>47</td>
<td>All ≤5 cm, number ≤3</td>
<td>Median, 3.1</td>
<td>91.5</td>
<td>51.1</td>
<td>63.8</td>
<td>Median, 63</td>
<td>38.3</td>
<td>83.0/14.9/2.1</td>
<td>OS, overall PFS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>46</td>
<td></td>
<td>Median, 2.8</td>
<td>84.8</td>
<td>32.6</td>
<td>82.6</td>
<td>Median, 64.5</td>
<td>34</td>
<td>87.0/13.0/-</td>
<td>OS, overall PFS</td>
<td>ND</td>
</tr>
<tr>
<td>Di Costanzo et al. 2015 (Italy)</td>
<td>RFA</td>
<td>70</td>
<td>Within Milan criteria</td>
<td>2.55</td>
<td>90.0</td>
<td>90.0</td>
<td>75.7</td>
<td>Median, 70</td>
<td>Mean, 42.8</td>
<td>90.0/10.0/-</td>
<td>OS, local PFS</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>LA</td>
<td>70</td>
<td></td>
<td>2.62</td>
<td>87.1</td>
<td>78.6</td>
<td>67.1</td>
<td>Median, 70</td>
<td>Mean, 42.2</td>
<td>90.0/10.0/-</td>
<td>OS, local PFS</td>
<td>1.4</td>
</tr>
<tr>
<td>Giorgio et al. 2011 (Italy)</td>
<td>PEI</td>
<td>143</td>
<td>Single ≤3 cm</td>
<td>2.27</td>
<td>100</td>
<td>100</td>
<td>71.3</td>
<td>72</td>
<td>37</td>
<td>52.4/47.6/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>142</td>
<td></td>
<td>2.34</td>
<td>100</td>
<td>100</td>
<td>73.9</td>
<td>70</td>
<td>37</td>
<td>49.3/50.7/-</td>
<td>OS</td>
<td>ND</td>
</tr>
<tr>
<td>Kim et al. 2021 (South Korea)</td>
<td>PBT</td>
<td>72</td>
<td>All &lt;3 cm, number ≤2</td>
<td>Median, 1.2</td>
<td>93.1</td>
<td>100</td>
<td>84.7</td>
<td>Median, 60</td>
<td>51.6</td>
<td>97.2/2.8/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>72</td>
<td></td>
<td>Median, 1.2</td>
<td>91.7</td>
<td>100</td>
<td>81.9</td>
<td>Median, 61.5</td>
<td>50.8</td>
<td>97.2/2.8/-</td>
<td>OS, overall PFS, local PFS</td>
<td>1.4</td>
</tr>
<tr>
<td>Koda et al. 2001 (Japan)</td>
<td>TACE + PEI</td>
<td>26</td>
<td>All &lt;3 cm, number ≤3</td>
<td>2.0</td>
<td>61.5</td>
<td>100</td>
<td>53.8</td>
<td>66.2</td>
<td>29</td>
<td>73.1/19.2/77</td>
<td>OS</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>26</td>
<td></td>
<td>1.9</td>
<td>57.7</td>
<td>100</td>
<td>69.2</td>
<td>66.4</td>
<td>31.2</td>
<td>53.8/30.8/15.4</td>
<td>OS</td>
<td>0</td>
</tr>
<tr>
<td>Lencioni et al. 2003 (Italy)</td>
<td>RFA</td>
<td>52</td>
<td>Within Milan criteria</td>
<td>2.8</td>
<td>76.9</td>
<td>88.5</td>
<td>69.2</td>
<td>67</td>
<td>22.9</td>
<td>86.5/13.5/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>50</td>
<td></td>
<td>2.8</td>
<td>62.0</td>
<td>84.0</td>
<td>60.0</td>
<td>69</td>
<td>22.4</td>
<td>70.0/30.0/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td>Lin et al. 2004 (Taiwan)</td>
<td>RFA</td>
<td>52</td>
<td>All 1-4 cm, number ≤3</td>
<td>2.9</td>
<td>73.1</td>
<td>71.2</td>
<td>67.3</td>
<td>62</td>
<td>24.5</td>
<td>78.8/21.2/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>52</td>
<td></td>
<td>2.8</td>
<td>76.9</td>
<td>73.1</td>
<td>65.4</td>
<td>59</td>
<td>23.8</td>
<td>75.0/25.0/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td>Lin et al. 2005 (Taiwan)</td>
<td>RFA</td>
<td>62</td>
<td>All ≤3 cm, number ≤3</td>
<td>2.5</td>
<td>79.0</td>
<td>100</td>
<td>64.5</td>
<td>61</td>
<td>28</td>
<td>74.2/25.8/-</td>
<td>OS, overall PFS, local PFS</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>62</td>
<td></td>
<td>2.3</td>
<td>79.0</td>
<td>100</td>
<td>62.9</td>
<td>60</td>
<td>26</td>
<td>75.8/24.2/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PAI</td>
<td>63</td>
<td></td>
<td>2.3</td>
<td>76.2</td>
<td>100</td>
<td>66.7</td>
<td>63</td>
<td>26</td>
<td>71.4/28.6/-</td>
<td>OS, overall PFS, local PFS</td>
<td>0</td>
</tr>
<tr>
<td>Mizuki et al. 2010 (Japan)</td>
<td>PEI</td>
<td>14</td>
<td>All 2-4 cm, number ≤3</td>
<td>2.64</td>
<td>78.6</td>
<td>ND</td>
<td>50.0</td>
<td>63.6</td>
<td>33</td>
<td>ND</td>
<td>OS, overall PFS</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TACE + PEI</td>
<td>13</td>
<td></td>
<td>2.64</td>
<td>61.5</td>
<td>ND</td>
<td>69.2</td>
<td>65.8</td>
<td>39.7</td>
<td>ND</td>
<td>OS, overall PFS</td>
<td>0</td>
</tr>
<tr>
<td>Study name (Country)</td>
<td>Arm</td>
<td>No. of patients</td>
<td>Inclusion criteria for tumor</td>
<td>Main tumor size* (cm)</td>
<td>Single HCC (%)</td>
<td>HCC ≤3 cm (%)</td>
<td>Male sex (%)</td>
<td>Age* (years)</td>
<td>Follow-up duration† (months)</td>
<td>CTP class A/B/C (%)</td>
<td>Outcome</td>
<td>Major complications‡ (%)</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----</td>
<td>----------------</td>
<td>----------------------------</td>
<td>----------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>----------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Paul et al. 2020 (India)</td>
<td>PAI</td>
<td>26</td>
<td>All ≤5 cm, number ≤5</td>
<td>2.7</td>
<td>80.8</td>
<td>66.7</td>
<td>76.9</td>
<td>53.5</td>
<td>13</td>
<td>73.1/26.9/-</td>
<td>OS</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>29</td>
<td></td>
<td>2.7</td>
<td>89.7</td>
<td>57.6</td>
<td>69.0</td>
<td>54.5</td>
<td>15</td>
<td>62.1/37.9/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peng et al. 2012 (China)</td>
<td>TACE + RFA</td>
<td>69</td>
<td>Within Milan criteria</td>
<td>2.1</td>
<td>94.2</td>
<td>59.4</td>
<td>85.5</td>
<td>57.5</td>
<td>39.2</td>
<td>87.0/13.0/-</td>
<td>OS, overall PFS</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>70</td>
<td></td>
<td>2.1</td>
<td>92.9</td>
<td>65.7</td>
<td>78.6</td>
<td>55.1</td>
<td>33.6</td>
<td>84.3/15.7/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shibata et al. 2009 (Japan)</td>
<td>TACE + RFA</td>
<td>46</td>
<td>All ≤3 cm, number ≤3</td>
<td>1.7</td>
<td>ND</td>
<td>100</td>
<td>67.4</td>
<td>67.2</td>
<td>30.4</td>
<td>69.6/30.4/-</td>
<td>OS, overall PFS, local PFS</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>43</td>
<td></td>
<td>1.6</td>
<td>ND</td>
<td>100</td>
<td>76.7</td>
<td>69.8</td>
<td>30.4</td>
<td>76.7/23.3/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shina et al. 2005 (Japan)</td>
<td>RFA</td>
<td>118</td>
<td>All ≤3 cm, number ≤3</td>
<td>ND</td>
<td>61.0</td>
<td>100</td>
<td>66.9</td>
<td>≤65/&gt;65, n=44/74</td>
<td>34.8</td>
<td>74.6/25.4/-</td>
<td>OS, overall PFS, local PFS</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>114</td>
<td></td>
<td>ND</td>
<td>52.6</td>
<td>100</td>
<td>76.3</td>
<td>≤65/&gt;65, n=41/73</td>
<td>37.2</td>
<td>72.0/28.0/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsai et al. 2008 (Taiwan)</td>
<td>PAI</td>
<td>70</td>
<td>All ≤4 cm, number ≤3</td>
<td>2.2</td>
<td>84.3</td>
<td>ND</td>
<td>67.1</td>
<td>65</td>
<td>Mean, 43</td>
<td>80.0/20.0/-</td>
<td>OS, local PFS</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>PEI</td>
<td>55</td>
<td></td>
<td>2.2</td>
<td>85.5</td>
<td>ND</td>
<td>74.5</td>
<td>66</td>
<td>Mean, 43</td>
<td>70.9/29.1/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietti Violi et al. 2018 (France and Switzerland)</td>
<td>MWA</td>
<td>70</td>
<td>All ≤4 cm, number ≤3</td>
<td>1.8</td>
<td>62.0</td>
<td>ND</td>
<td>83.1</td>
<td>Median, 68</td>
<td>26</td>
<td>80.3/19.7/-</td>
<td>OS, local PFS</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>73</td>
<td></td>
<td>1.8</td>
<td>63.0</td>
<td>ND</td>
<td>84.9</td>
<td>Median, 65</td>
<td>25</td>
<td>72.6/27.4/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wang et al. 2015 (China)</td>
<td>CA</td>
<td>180</td>
<td>All ≤4 cm, number ≤2</td>
<td>ND</td>
<td>89.4</td>
<td>ND</td>
<td>77.8</td>
<td>53.9</td>
<td>25</td>
<td>67.2/32.8/-</td>
<td>OS, overall PFS, local PFS</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>RFA</td>
<td>180</td>
<td></td>
<td>ND</td>
<td>95.0</td>
<td>ND</td>
<td>83.3</td>
<td>53.3</td>
<td>25</td>
<td>60.6/39.4/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaitoun et al. 2021 (Egypt)</td>
<td>TACE</td>
<td>84</td>
<td>Single 3-5 cm</td>
<td>3.6</td>
<td>100</td>
<td>ND</td>
<td>61.9</td>
<td>51.3</td>
<td>Mean, 19</td>
<td>84.5/15.5/-</td>
<td>OS</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>MWA</td>
<td>92</td>
<td></td>
<td>3.9</td>
<td>100</td>
<td>ND</td>
<td>54.3</td>
<td>53.8</td>
<td>Mean, 21</td>
<td>84.8/15.2/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TACE + MWA</td>
<td>89</td>
<td></td>
<td>3.7</td>
<td>100</td>
<td>ND</td>
<td>58.4</td>
<td>52.1</td>
<td>Mean, 24</td>
<td>89.9/10.1/-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; CTP, Child-Turcotte-Pugh; RFA, radiofrequency ablation; MWA, microwave ablation; PEI, percutaneous ethanol injection; LA, laser ablation; TACE, trans-arterial chemoembolization; PBT, proton beam therapy; PAI, percutaneous acetic acid injections; CA, cryoablation; OS, overall survival; PFS, progression-free survival; ND, not described in the text.

*Main tumor size and age are presented as mean values, unless otherwise stated. †Follow-up durations are presented as median values, unless otherwise stated. ‡Results for major complications were based on the criteria defined and set by the individual studies: (for example) bowel perforation, hemorrhage, infection, third space fluid collection needing intervention, pneumothorax needing chest tube, needle-track tumor seeding, hepatic failure, and procedure-related death.
cutaneous ethanol (PEI; S85 in 9\(^{5,24,27,29,33,36-38}\)) and acetic acid injections (PAI; 159 in 3\(^{29,30,38}\)) and TACE+PEI (39 in 2\(^{36,37}\)).

A network map of the treatment relationships and comparisons is presented in Figure 2. Node size and line thickness were proportional to the number of included patients and number of trials, respectively. Characteristics of the 19 studies included in the network are summarized in Table 1. The percentage of patients with solitary HCC ranged from 56.9% to 100%, while the percentage of patients with multifocal HCCs ranged from 0% to 43.1%. The number of tumors per patient in each study ranged from 1 to 4, and only eight patients in two trials, Shiina et al.\(^{33}\), 2005 and Paul et al.\(^{30}\), 2020, had >3 index lesions. Overall, 37.9% to 97.2% of patients were classified as Child-Turcotte-Pugh (CTP) class A, and 2.8% to 55.9% of patients were classified as CTP class B. All included trials reported OS as a study outcome; 11 included overall PFS (or TTP); and 11 included local PFS (or TTP). Local or overall PFS results were substituted for the corresponding TTP in seven and one studies, respectively.

**Risk of bias assessments**

Most studies were graded as low risk for the domains including randomization process, missing outcome data, measurement of outcome data, and selection of the reported result. Three studies reported the results of a modified intention-to-treat analysis, which were considered to be at high risk for domain deviations from intended interventions. Overall, the trials were considered to be at low risk for bias. Details of these assessments are presented in Figure 3 and Supplementary Figure 1.

**OS analysis**

The NMA included all RCTs in the OS analysis (Fig. 2A). Only TACE+RFA had significantly better OS than RFA when used for HCCs ≤5 cm (HR, 0.52; 95% CI, 0.33–0.82; Fig. 4A). Conversely, PEI (1.51, 1.16–1.96) and PAI (1.99, 1.30–3.06) resulted in poorer OS than RFA. No significant differences in OS were observed between RFA and the following treatments:

---

**Figure 1.** PRISMA flow diagram of the process of screening and selecting studies. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; HCC, hepatocellular carcinoma.
Figure 2. Network plots for (A) overall survival, (B) overall progression-free survival, and (C) local progression-free survival for direct comparisons of 19, 10, and 9 selected RCTs, respectively. Circle sizes reflect numbers of participants, while line widths reflect numbers of direct comparisons. The absence of a connecting line between two treatments indicates that there was no direct comparison. RCT, randomized controlled trial; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; PEI, percutaneous ethanol injection; MWA, microwave ablation; PBT, proton beam therapy; PAI, percutaneous acetic acid injection; LA, laser ablation; CA, cryoablation.

Figure 3. Evaluation of risk of bias in the randomized controlled trials.
TACE+MWA, CA, PBT, MWA, and LA. Probabilistic ranking metrics based on P-scores indicated that TACE+RFA (0.951) also ranked highest of all 11 treatment classes with respect to magnitude of treatment effect on OS, with a 61.4% probability of being the most effective option on the rankogram (Fig. 4A and Supplementary Fig. 2A). TACE+MWA and CA were the...
second-best and third-best treatments, respectively, and PAI had the highest probability (48.3%) of being the lowest ranked. Compared to RFA, no significant difference in OS was found with TACE (HR, 1.53; 95% CI, 0.74–3.16) despite having the second lowest P-score (0.279). The same trend in the ranking of treatments according to OS was observed for the extrapolated parametric survival NMA model for time-to-event (Fig. 5).

In pairwise comparisons within the complete network of 19 trials, TACE+RFA was significantly superior with respect to OS to every other treatment except TACE+MWA, CA, and PBT (Fig. 6). In addition, the four thermal or radiation therapies (i.e., CA, RFA, PBT, and MWA) were associated with significantly greater OS than PEI or PAI.

Overall PFS analysis

To evaluate overall PFS, we analyzed the relevant data from 10 studies involving eight different therapies in 1,477 patients (Fig. 2B). Compared to RFA alone, significant HRs for overall PFS were found for TACE+RFA (HR, 0.61; 95% CI, 0.42–0.88) and PAI (3.85; 1.25–11.79) (Fig. 4B). These two treatments were ranked in first and last place, respectively, based on P-score (0.964 and 0.030, respectively). The ranking profile was generally consistent with that of OS (Supplementary Fig. 2B). In addition, the seven pairwise treatment comparisons indicated a significant advantage of TACE+RFA over RFA, MWA, PEI, or PAI in improving overall PFS (Fig. 6).

Local PFS analysis

Local PFS analysis comprised nine studies investigating eight therapeutic options in 1,394 patients (Fig. 2C). No treatment modalities detected a local PFS benefit when compared with RFA (Fig. 4C). When ordered by P-score, CA and TACE+RFA were ranked in first and second place, respectively. The rankogram also indicated that CA was the most effective treatment in prolonging local PFS duration (Supplementary Fig. 2C). In terms of pairwise comparisons, all treatment pairs other than PAI and PEI yielded comparable rates of local PFS (Supplementary Fig. 3).

Adverse events

A total of 16 studies reported major adverse events among 2,304 patients undergoing 11 distinct therapies. Due to the lack of a consistent definition for major adverse events across the studies, a quantitative assessment could not be made. The observed prevalence of major complications ranged from 0% to 7.7%, depending on the specific criteria used in each study.

Regarding treatment modalities, RFA was associated with a major complication rate that varied from 0% to 6.9% across 12 studies. Post-RFA complications included lung-related problems such as hemоторax, pneumоторax, and pleural effusion, in addition to hemorrhage, hepatic decompensation (manifesting as ascites or jaundice), malignant cell seeding, infection, and hepatic infarct/necrosis.

The major complication rates for other treatment modalities were as follows: PEI, 0–2.9% (eight studies); PAI, 0–7.7% (three studies); MWA, 2.2–2.8% (two studies); and TACE+RFA, 2.2–2.9% (two studies). Notable adverse events associated by modality were as follows: PEI (one death due to bowel infarction, neoplastic seeding, and liver abscess), PAI (two deaths resulting from hepatic necrosis and tumor rupture, tumor seeding, and gross hematuria), MWA (hemorrhage and tumor seeding), and TACE+RFA (hepatic decompensation and segmental hepatic infarction). The remaining six treatment modalities were each investigated in a single study. A comprehensive description of adverse events is presented.
Overall progression-free survival

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OS HR (95% CI)</th>
<th>OS 95% CI</th>
<th>PFS HR (95% CI)</th>
<th>PFS 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACE+RFA</td>
<td>0.67 (0.43-1.11)</td>
<td>0.61 (0.42-0.88)</td>
<td>0.62 (0.37-1.02)</td>
<td>0.58 (0.34-0.99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TACE+MWA</td>
<td>0.75 (0.24-2.32)</td>
<td>0.82 (0.25-2.70)</td>
<td>0.89 (0.56-1.40)</td>
<td>0.83 (0.51-1.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>0.49 (0.65-1.18)</td>
<td>0.46 (0.65-1.18)</td>
<td>0.46 (0.65-1.18)</td>
<td>0.46 (0.65-1.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFA</td>
<td>0.30 (0.58-1.48)</td>
<td>0.41 (0.51-1.73)</td>
<td>0.41 (0.51-1.73)</td>
<td>0.41 (0.51-1.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBT</td>
<td>0.94 (0.55-1.59)</td>
<td>0.94 (0.55-1.59)</td>
<td>0.94 (0.55-1.59)</td>
<td>0.94 (0.55-1.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MWA</td>
<td>0.89 (0.34-2.37)</td>
<td>0.89 (0.34-2.37)</td>
<td>0.89 (0.34-2.37)</td>
<td>0.89 (0.34-2.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.89 (0.31-2.50)</td>
<td>0.89 (0.31-2.50)</td>
<td>0.89 (0.31-2.50)</td>
<td>0.89 (0.31-2.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEI</td>
<td>0.56 (0.34-0.92)</td>
<td>0.56 (0.34-0.92)</td>
<td>0.56 (0.34-0.92)</td>
<td>0.56 (0.34-0.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TACE+PAI</td>
<td>0.59 (0.23-2.13)</td>
<td>0.59 (0.23-2.13)</td>
<td>0.59 (0.23-2.13)</td>
<td>0.59 (0.23-2.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TACE</td>
<td>0.29 (0.07-1.28)</td>
<td>0.29 (0.07-1.28)</td>
<td>0.29 (0.07-1.28)</td>
<td>0.29 (0.07-1.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFA</td>
<td>0.49 (0.15-1.55)</td>
<td>0.49 (0.15-1.55)</td>
<td>0.49 (0.15-1.55)</td>
<td>0.49 (0.15-1.55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI</td>
<td>0.77 (0.33-1.78)</td>
<td>0.77 (0.33-1.78)</td>
<td>0.77 (0.33-1.78)</td>
<td>0.77 (0.33-1.78)</td>
</tr>
</tbody>
</table>

**Figure 6.** League table showing hazard ratios (HRs) for pairwise comparisons of overall survival (OS) and overall progression-free survival (PFS) between treatments. Comparisons should be read from left to right. HRs (95% confidence intervals [CI]) for comparisons are in the cells shared by the column-defining and row-defining interventions. Numbers written in bold are statistically significant. For OS, an HR of <1 favors the row-defining treatment. For overall PFS, an HR of <1 favors the column-defining treatment. NA, not applicable; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; MWA, microwave ablation; CA, cryoablation; PBT, proton beam therapy; LA, laser ablation; PEI, percutaneous ethanol injection; PAI, percutaneous acetic acid injection.

**Subgroup analysis**

Among the 19 RCTs, seven studies included a total of 1,128 patients with HCC nodules ≤3 cm in diameter undergoing six distinct therapies. These studies were included in a network created to analyze HRs for OS. No significant differences in OS (HR [95% CI], 0.67 [0.21–2.20]; Supplementary Fig. 4A) were found between the combined TACE+RFA and RFA alone groups, although survival was slightly better in the TACE+RFA group. Similar results were obtained for both overall PFS in four studies with 652 patients (0.84 [0.51–1.41]) and local PFS in three studies with 420 patients (0.63 [0.25–1.59]), as shown in Supplementary Figure 4B and 4C.

**Assessment of transitivity and inconsistency**

Overall, the transitivity assumption was not challenged, as there were no significant differences in the baseline parameters examined to evaluate its plausibility (Supplementary Fig. 5). There were also no significant differences for either OS or local PFS in terms of inconsistencies between direct and indirect estimates in the node-splitting analysis within the closed loop in the evidence network (PEI-PAI-RFA) (Supplementary Table 4).

**DISCUSSION**

The above analysis based on the published outcomes of RCTs of loco-regional therapies revealed that combined TACE and RFA occupies the first position in a ranking of non-surgical treatments of early HCCs meeting the Milan criteria. The analysis based on the integrated scores for OS and PFS showed that combined TACE and RFA is superior to all the other mono- or dual-therapeutic options, including TACE and RFA individually. In addition, all single interventions, other
than PEI and PAI, the oldest techniques for the treatment of HCC, had comparable survival- and progression-related efficacy compared to RFA, the standard strategy according to the current evidence-based management of this disease stage.

The RCTs and meta-analyses had limitations in terms of sample size, study quality, and statistical power. Nonetheless, the cumulative evidence from these studies indicated that TACE+RFA is superior to RFA alone in terms of survival and/or recurrence, particularly for studies including HCCs ≥3 cm, and that it was no associated with any significant major complications. \(^{39,40}\) It is likely that the benefits of TACE followed as soon as possible by RFA derive from (1) improved control of microsatellite lesions due to the larger treated area\(^ {41}\); (2) reduced vascular cooling effect that can lead to incomplete ablation; (3) prevention of portal invasion of the tumor by hepatic arterial and portal venous flows occluded by embolic materials; (4) boosting of the thermal effect by chemotherapeutic drugs and ischemic edema; and (5) optimization of heat diffusion by the disruption of intratumoral septa. \(^ {42,43}\) Our investigation of nodules up to 5 cm in diameter also concluded that this combination was more effective than RFA, MWA, or LA therapy alone. Furthermore, given the discrepancy between the overall (positive) and local (negative) PFS results of TACE+RFA, our results suggest that the addition of TACE may prolong OS, compared with RFA alone, by targeting microsatellites together around the target lesion. Surprisingly, our analysis revealed no beneficial effect on OS when adding TACE to MWA, although this combination was comparable to TACE+RFA based on pairwise comparisons. A retrospective study in the U.S. comparing 38 patients treated with TACE+RFA and 51 treated with TACE+MWA for HCCs of varying sizes yielded similar safety and efficacy outcomes; \(^ {44}\) the ranking of these combination therapies in term of safety and efficacy needs to be confirmed by further relevant RCTs.

Our findings indicate that TACE+RFA deserves primary consideration for curative control of early HCCs, apart from ultrasound-invisible HCCs requiring radiographic guidance for curative RFA treatment by TACE-induced iodized oil retention. \(^ {45}\) Subgroup analyses based on sub-networks of RCTs explicitly reporting data restricted to patients with HCC nodules ≤3 cm in diameter reported no significant differences in local PFS, overall PFS, or OS between combined TACE+RFA and RFA alone (Supplementary Fig. 4). The study from Japan of Shibata et al. \(^ {32}\) included in our network illustrates this point. Nevertheless, these findings support the idea that RFA monotherapy is adequate for treating small (≤3 cm) HCCs, when patient convenience, hospital stay, and medical costs are taken into account. \(^ {36}\) Therefore, combination therapy may be more useful for medium-sized (3–5 cm) HCCs, as complete necrosis is difficult to achieve with RFA alone, particularly in infiltrating tumor types. \(^ {36}\) This conclusion is consistent with the current recommendations stipulated in the recently updated Korean guidelines. \(^ {2}\)

For imaging-guided percutaneous ablation techniques, such as RFA, MWA, CA, and LA, our direct and indirect comparison data revealed no significant differences in both survival and progression endpoints among the mono-modalities; this is consistent with reports of individual paired comparative studies based on various design types. \(^ {13,23,25,26}\) Each technique has its own advantages and limitations compared with the traditional RFA reference: for instance, MWA can produce larger coagulation areas than RFA with shorter ablation times; it is also less sensitive to the heat-sink effect and hence achieves adequate ablation of nodules close to large vessels; however, it is contraindicated for treating nodules at high-risk locations as well as subcapsular nodules. \(^ {47,48}\) In spite of technical difficulties, LA is safer for treating nodules at difficult locations, as well as multiple lesions in one session, as it uses multiple fibers and spares the uninvolved hepatic parenchyma. \(^ {49}\) Lastly, CA, which is much less painful than RFA, has the advantage of protecting better against vascular and biliary injuries by monitoring the extent of ablation during the procedure, despite producing larger ablation volumes with multiple probes. \(^ {50}\) Our findings provide prognostic evidence justifying a tailored approach in which other liver-directed thermal ablation procedures are placed alongside RFA as standard-of-care for maximizing treatment efficacy and minimizing procedure-related complications in patients considered to be candidates for HCC ablation.

Several HCC studies have reported good outcomes with hypo-fractionated PBT having a finite range of energy deposition and thus low rates of hepatic and gastrointestinal toxicity relative to photon beam therapy. \(^ {8,51}\) A recent U.S. registry study of 918 patients with T1 or T2 HCC suggested that PBT may have superior survival outcomes compared to SBRT. \(^ {52}\) The latter is another conformal external technique, which was not included in our NMA because of the absence of relevant RCTs. However, a meta-analysis of 70 non-comparative observational studies suggesting that the two radio-
therapies have similar efficacy with respect to OS and overall and local PFSs warrants prospective corroboration. Based on our NMA findings and the existing evidence, PBT (or SBRT if PBT is not feasible) may be considered alternative treatments for percutaneous options including RFA, particularly for nodules considered to be at high risk, with limited accessibility, or invisible under ultrasound guidance. Unlike injection therapies with ethanol or acetic acid, which have few clinical applications and have been found to be inferior to RFA in numerous comparisons, TACE, with a broader spectrum of indications, remains the first choice in early-stage cases that are neither suitable nor appropriate for local ablation when based on the stage migration strategy. Pairwise direct and indirect evidence from our NMA may justify a second-best role of TACE monotherapy as an alternative next-line option for HCCs below the intermediate stage. It should also not be overlooked that several matched and non-matched studies of within-Milan HCCs have found that TACE has comparable effects to therapeutic ablation and radiation on tumors and patients.

Apart from the inherent limitations of collecting aggregate patient data from completed studies, some limitations of this work should be acknowledged. First, we could not include an estimate of safety profile as an outcome of interest in the present NMA because of the heterogeneous criteria for, and definitions of, procedure-related adverse events across the studies. However, almost all the intervention arms in all the RCTs had ≤5% serious adverse events (Table 1). Second, a few patients with CTP class C liver function or >3 target lesions who are not usually good candidates for loco-regional therapies but were included in our analysis, albeit rarely, may have affected the interpretation of survival and efficacy outcomes (Table 1). Lastly, in most (n=12) of the included RCTs, the inclusion criteria specified HCCs up to 5 cm, which prevented us from enrolling only ideal candidates with tumors ≤3 cm. However, we were able to determine the outcomes for HCC lesions ≤3 cm through a subgroup analysis of seven studies.

In conclusion, in this loco-regional therapy-based NMA, we found that TACE+RFA was ranked highest with regard to both survival and progression outcomes in the treatment of patients with early HCCs ≤5 cm. Equivalent outcomes are likely for HCCs within the Milan criteria that can be optimally treated by thermal ablation using a variety of possible energy sources or PBT. Further evidence concerning specific indications for the individual modalities is urgently needed to develop precisely tailored strategies.

Authors’ contribution
Hi Kim, J An, and JH Shim contributed to the study concept and design, acquisition, analysis and interpretation of data, verification of the underlying data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. S Han contributed to the statistical analysis, verification of the underlying data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. All authors confirm that they had full access to all the data in the study and accept responsibility for submission of this manuscript.

Acknowledgements
This study was supported by grants from the National Research Foundation of Korea funded by the Ministry of Science and ICT (NRF-2022R1A2C3008956 and RS-2022-00166674), the Research Supporting Program of The Korean Association for the Study of the Liver and The Korean Liver Foundation (KASLKF2018-05) and Asan Institute for Life Sciences, Asan Medical Center (2022IP0046).

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


45. Park BJ, Byun JH, Jin YH, Won HJ, Shin YM, Kim KW, et al. CT-guided radiofrequency ablation for hepatocellular carcinomas that were undetectable at US: therapeutic effectiveness and
Prognostic role of computed tomography analysis using deep learning algorithm in patients with chronic hepatitis B viral infection

Jeongin Yoo1*, Heejin Cho2*, Dong Ho Lee1,3, Eun Ju Cho2, Ijin Joo1,2,4, and Sun Kyung Jeon1

1Department of Radiology, Seoul National University Hospital, Seoul; 2Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul; 3Department of Radiology, Seoul National University College of Medicine, Seoul; 4Institute of Radiation Medicine, Seoul National University Medical Research Center, Seoul, Korea

Graphical Abstract

CT analysis of body composition and organ volume using deep learning algorithm:
Prognostic role in patients with chronic hepatitis B viral infection

Study Highlights

- Previous studies have shown the usefulness of deep learning-based automated CT analysis for opportunistic screening of various diseases. Therefore, this study aimed to evaluate comprehensive prognosis through organ volumes and body composition measurements obtained from CT data using deep learning-based fully automated organ segmentation algorithm in patients with chronic hepatitis B. A larger standardized spleen volume was significantly associated with HCC, hepatic decompensation, and occurrence of DM. A higher abdominal VAT index was correlated with the development of DM, while a higher SAT index was correlated with increased OS.
INTRODUCTION

Chronic hepatitis B (CHB) remains an important global health problem with significant morbidity and mortality despite vaccination and effective antiviral treatment. The risk of progression to cirrhosis and hepatocellular carcinoma (HCC) in patients with CHB is variable and is affected by the host’s immune response. The 5-year cumulative incidence of cirrhosis ranges from 8% to 20% in patients with untreated CHB and the 5-year cumulative risk of hepatic decompensation among those with cirrhosis is 20%. The annual risk of HCC in patients with cirrhosis has been reported to be 2–5%. The risk factors for CHB progressing to cirrhosis or HCC include not only host-related or viral factors, but also social-environmental factors (e.g., alcohol consumption, metabolic syndrome, diabetes mellitus [DM], obesity, and smoking). There have been efforts over decades to predict clinical outcomes in patients with CHB for proper and timely management such as GAGHCC, CU-HCC, REACH-B, or PAGE-B for predicting HCC development, hepatic decompensation and overall survival (OS) and model for end-stage liver disease, hepatic venous pressure gradient, and albumin for predicting hepatic decompensation and OS in previous literatures. Deep learning-based computed tomography (CT) metric

**Background/Aims:** The prediction of clinical outcomes in patients with chronic hepatitis B (CHB) is paramount for effective management. This study aimed to evaluate the prognostic value of computed tomography (CT) analysis using deep learning algorithms in patients with CHB.

**Methods:** This retrospective study included 2,169 patients with CHB without hepatic decompensation who underwent contrast-enhanced abdominal CT for hepatocellular carcinoma (HCC) surveillance between January 2005 and June 2016. Liver and spleen volumes and body composition measurements including subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and skeletal muscle indices were acquired from CT images using deep learning-based fully automated organ segmentation algorithms. We assessed the significant predictors of HCC, hepatic decompensation, diabetes mellitus (DM), and overall survival (OS) using Cox proportional hazard analyses.

**Results:** During a median follow-up period of 103.0 months, HCC (n=134, 6.2%), hepatic decompensation (n=103, 4.7%), DM (n=432, 19.9%), and death (n=120, 5.5%) occurred. According to the multivariate analysis, standardized spleen volume significantly predicted HCC development (hazard ratio [HR] = 1.01, P = 0.025), along with age, sex, albumin and platelet count. Standardized spleen volume (HR = 1.01, P < 0.001) and VAT index (HR = 0.98, P = 0.004) were significantly associated with hepatic decompensation along with age and albumin. Furthermore, VAT index (HR = 1.01, P = 0.001) and standardized spleen volume (HR = 1.01, P = 0.001) were significant predictors for DM, along with sex, age, and albumin. SAT index (HR = 0.99, P = 0.004) was significantly associated with OS, along with age, albumin, and MELD.

**Conclusions:** Deep learning-based automatically measured spleen volume, VAT, and SAT indices may provide various prognostic information in patients with CHB. (Clin Mol Hepatol 2023;29:1029-1042)

**Keywords:** Chronic hepatitis B; Hepatocellular carcinoma; Liver cirrhosis; Diabetes mellitus; Survival

**Corresponding author:** Dong Ho Lee
Department of Radiology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-2584; Fax: +82-2-743-6385, E-mail: dhlee.rad@gmail.com
http://orcid.org/0000-0001-8983-851X

*J Yoo and H Cho contributed equally as co-first authors.

**Editor:** Yong Eun Chung, Yonsei University College of Medicine, Korea

**Received:** Jun. 1, 2023 / **Revised:** Aug. 8, 2023 / **Accepted:** Aug. 27, 2023

**Abbreviations:**
CHB, chronic hepatitis B; CI, confidence interval; CT, computed tomography; DM, diabetes mellitus; HCC, hepatocellular carcinoma; HR, hazard ratio; OS, overall survival

analysis provides three-dimensional organ volumetric parameters and body composition measurements.\textsuperscript{11,12} Furthermore, previous investigations\textsuperscript{13,14} showed that fully automated quantitative tissue composition analysis using CT scans may predict future serious adverse events and add opportunistic value to CT scans performed for other indications. Considering the promising results of previous studies, we surmised that body composition analysis and organ volume measurement using liver CT images may also provide prognostic information regarding the risk of HCC occurrence or development of hepatic decompensation in patients with compensated CHB.

Therefore, the purpose of this study was to evaluate the prognostic value of CT analysis using deep learning algorithm in patients with CHB.

**MATERIALS AND METHODS**

This study was approved by our institutional review board (IRB No.: 2108-143-1246) and the requirement for signed informed consent was waived owing to the retrospective design.

**Patients**

The inclusion criteria were: 1) patients aged between 19 and 85 years, 2) patients who were diagnosed with CHB, and 3) patients having both a contrast-enhanced abdominal CT scan and complete medical records, including liver function tests, between January 2006 and June 2016 (Fig. 1). According to the inclusion criteria, 6,140 consecutive patients were assessed for eligibility. The exclusion criteria were: 1) previous history of HCC (n=3,546), 2) previous history of hepatic decompensation (n=90), 3) previous history of solid organ transplantation (n=48), 4) poor image quality of abdominal CT scan, when reviewed by a radiologist (n=84), and 5) history of malignancies other than HCC (n=39). Finally, 2,333 patients constituted the study population.

**CT image acquisition**

All patients underwent abdominal CT scans, including the arterial and portal venous phase, using various types of CT scanners owing to the retrospective study design. CT scanning was performed using the following parameters: tube voltage, 90–120 kVp according to the scanner type; tube current-time products, 100–300 mAs; rotation time, 0.5 s; pitch, 0.6–1.2; and slice thickness, 3 mm. Iobitridol (Xenetix 350;...
Guerbet, Aulnay-sous-Bois, France) was intravenously injected at a dose of 520 mg/kg body weight using a power injector (Stellant; Bayer AG, Berlin, Germany) for 30 s at a rate of 2–5 mL/s according to body weight, followed by a 20–30-mL saline flush. Using the bolus tracking method, arterial phase scans were started 17–23 s after the enhancement threshold (100–150 HU) was reached in the descending thoracic aorta. For portal venous phase scans, a fixed delay of 60–75 s was used.

Body composition and organ volume analysis using CNN

CT images were processed in an automated analysis software program using convolutional neural network (CNN) (DeepCatch and MEDIP; MedicalIP Co. Ltd., Seoul, Korea; http://www.medicalip.com). After uploading portal venous phase CT images to commercially available segmentation software (MEDIP Deep Catch v1.0.0.0; MedicalIP Co. Ltd.), a three-dimensional U-Net automatically provided volumetric segmentation of body components into seven classes (skin, subcutaneous fat, muscle, visceral fat, bone, internal organs and vessels, and the central nervous system) (Fig. 2). The average dice scores for muscles, visceral fat, and for subcutaneous fat were 96.8–99.2%, 95.1–98.9%, and 97.1–99.7%, respectively, in the domestic validation sets. After segmentation, the software also provided automatic segmentation and labeling of the body composition area at the L3-level cross-sectional image. An experienced radiologist, who was blinded to the patients’ clinicopathological information, confirmed the results of the segmentation. Subsequently, L3-level sectional area (cm²) of the skeletal muscle, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) were normalized to the height (m)0.725 and were labeled as skeletal muscle, SAT, and VAT indices, respectively. Additionally, three-dimensional segmentation of the liver and spleen was performed and the organ volumes were automatically calculated. Spleen and liver volumes were normalized to the body surface area (m²), which was calculated as body weight (kg)0.425 × height (cm)0.725 × 0.007184.

Endpoints

The primary endpoint was OS and the secondary endpoints were liver-related (i.e., development of HCC and decompensation) and metabolic outcomes (i.e., development of DM). We assessed the cumulative incidence of the occurrence of HCC, hepatic decompensation, DM, and death. Development of HCC was determined histopathologically in patients who underwent surgical resection or percutaneous biopsy, or based on imaging features categorized into “LR-5” according to the Liver Imaging Reporting and Data System or “definite HCC” according to the 2022 Korean Liver Cancer Association and National Cancer Center Korea practice guidelines, in patients without histopathologic analysis. The development of hepatic decompensation was defined as the occurrence of variceal bleeding, spontaneous bacterial peritonitis, hepatic encephalopathy, or hepatorenal syndrome. The development of DM was determined based on medical records. OS was calculated as the interval between the day of the baseline liver CT and death or the last follow-up date. The survival data of the study population was acquired from the national registry data from the Korean Ministry of Interior and Safety. The data cut-off date was January 31, 2022.

Figure 2. Fully automated body composition and organ volume analysis using convolutional neural network. (A) A three-dimensional U-Net automatically provided volumetric segmentation of body components into seven classes (skin, subcutaneous fat, muscle, visceral fat, bone, internal organs and vessels, and the central nervous system). (B) An axial image shows the results of segmentation, which are overlaid on orthogonal cross-sectional images at the L3 vertebral-body level. Pink, yellow, green, and purple colors indicate skeletal muscle, subcutaneous fat, visceral fat, and internal organs, respectively. (C) An axial image shows the results of organ segmentation of the liver (yellow) and spleen (red), which are overlaid on the CT image. (D) Three-dimensional segmentation of the liver and spleen was performed. CT, computed tomography.
Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows version 27.0 (IBM Corp., Armonk, NY, USA), SAS version 9.3 (SAS Institute Inc., Cary, NC, USA), and MedCalc Statistical Software version 18.9.1 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019). Univariate and multivariate Cox proportional hazards logistic regression analyses were performed to identify significant predictors of each outcome. All variables with p-values less than 0.05, in univariate analyses, were included in the multivariate analysis using stepwise selection. The Kaplan–Meier method was used for estimation of the cumulative incidence of each outcome. The optimal cut-off values of body composition and organ volume measurements for predicting each outcome were determined using the minimal P-value approach based on log-rank test statistics. Statistical significance was set at a P-value <0.05.

RESULTS

Patient characteristics

Baseline characteristics of the 2,333 patients (Male: Female=1,396:937; median age, 52.0 years [interquartile range (IQR), 45.0–59.0]) are summarized in Table 1. Among them, 164 patients had DM at the time of enrollment. For all patients, body composition data including skeletal muscle, VAT, and SAT indices were successfully obtained from CT data analyzed by deep learning algorithm. Additionally, liver and spleen volumes of each patient were successfully acquired from their CT data via deep learning algorithm and standardized by dividing by body surface area.

Predictive factors for HCC development

During a median follow-up period of 103 months, HCC developed in 134 patients (5.7%, 134/2,333) with the following stages at diagnosis: Barcelona–Clinic Liver Cancer stage 0 (n=70), stage A (n=59), stage B (n=4), and stage C (n=1). The estimated the 1-, 5-, and 10-year cumulative incidences of HCC occurrence were 0.6%, 3.2%, and 6.1%, respectively. According to the multivariate analysis, standardized spleen volume was one of the significant predictive factors for HCC development (hazard ratio [HR]=1.01, 95% confidence interval [CI]=1.01–1.01, P = 0.025), along with age, sex, albumin level, and platelet count (Table 2). The optimal cut-off value for the

Table 1. Baseline characteristics of 2,333 patients with compensated chronic liver disease from CHB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.0 (45.0–59.0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1,396 (59.8)</td>
</tr>
<tr>
<td>Females</td>
<td>937 (40.2)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>164 (7.0)</td>
</tr>
<tr>
<td>No</td>
<td>2,169 (93.0)</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>27.0 (19.0–47.0)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>43.0 (41.0–45.0)</td>
</tr>
<tr>
<td>Total bilirubin level (mg/dL)</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>Prothrombin activity (INR)</td>
<td>1.03 (0.97–1.08)</td>
</tr>
<tr>
<td>Platelet count (K/mm$^3$)</td>
<td>195.0 (159.0–233.0)</td>
</tr>
<tr>
<td>Alpha fetoprotein (ng/mL)</td>
<td>2.7 (1.6–4.2)</td>
</tr>
<tr>
<td>APRI</td>
<td>0.35 (0.25–0.58)</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.41 (0.99–2.05)</td>
</tr>
<tr>
<td>MELD</td>
<td>7.0 (6.5–8.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>23.8 (21.8–26.0)</td>
</tr>
<tr>
<td>Standardized liver volume (mL/cm$^2$)</td>
<td>715.7 (641.8–802.4)</td>
</tr>
<tr>
<td>Standardized spleen volume (mL/cm$^2$)</td>
<td>99.1 (78.0–128.4)</td>
</tr>
<tr>
<td>Skeletal muscle index (cm$^2$/m$^2$)</td>
<td>47.1 (40.9–53.6)</td>
</tr>
<tr>
<td>Visceral adipose tissue index (cm$^2$/m$^2$)</td>
<td>25.3 (11.9–41.7)</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue index (cm$^2$/m$^2$)</td>
<td>48.0 (34.5–64.5)</td>
</tr>
<tr>
<td>Antiviral therapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,276 (54.7)</td>
</tr>
<tr>
<td>No</td>
<td>1,057 (45.3)</td>
</tr>
<tr>
<td>HBeAg</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,877 (80.5)</td>
</tr>
<tr>
<td>No</td>
<td>456 (19.5)</td>
</tr>
<tr>
<td>REACH-B</td>
<td>7.0 (6.0–10.0)</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range) or number (%). CHB, chronic hepatitis B viral infection; INR, international normalized ratio; IU, international unit; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4 index; MELD, model for end-stage liver disease; HBeAg, hepatitis B e antigen; REACH-B, risk estimate for hepatocellular carcinoma in chronic hepatitis B.
standardized spleen volume was set at 112.6 mL/m² to predict HCC development. The estimated 1-, 5-, and 10-year cumulative incidences of HCC development in 773 patients with standardized spleen volumes ≥112.6 mL/m² were 0.8%, 5.1%, and 9.9%, respectively, and were significantly higher than those in 1,396 patients with standardized spleen volumes <112.6 mL/m² which were 0.4%, 2.2%, and 3.9%, respectively (HR=1.71, 95% CI =1.21–2.42, P=0.002) (Fig. 3A).

**Predictive factors for the development of hepatic decompensation**

During the follow-up period, 103 patients (4.4%, 103/2,333) experienced hepatic decompensation with the development of: ascites (n=59); hepatic encephalopathy (n=33); and variceal bleeding (n=11). The estimated 1-, 5-, and 10-year cumulative incidences for the development of hepatic decompensation were 0.7%, 2.8%, and 5.1%, respectively. On multivariate analysis, age and serum albumin level were significantly associated with the development of hepatic decompensation. In addition, standardized spleen volume (HR=1.01, 95% CI=1.01–1.01, P<0.001) and VAT index (HR=0.98, 95% CI=0.97–0.99, P=0.004) were also significant predictive factors for hepatic decompensation (Table 3). The optimal cut-off value of the standardized spleen volume was set at 145.74 mL/m² to predict hepatic decompensation. The estimated 1-, 5-, and 10-year cumulative incidences of hepatic decompensation in 329 patients with a standardized spleen volume ≥145.74 mL/m² was 0.7%, 2.5%, and 3.9%, respectively (HR=2.34, 95%

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>0.46</td>
<td>0.31–0.69</td>
</tr>
<tr>
<td>Age (per 1 year)</td>
<td>1.03</td>
<td>1.01–1.05</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>1.04</td>
<td>0.99–1.08</td>
</tr>
<tr>
<td>Antiviral therapy (yes)</td>
<td>1.46</td>
<td>1.05–2.03</td>
</tr>
<tr>
<td>HBeAg (positive)</td>
<td>1.16</td>
<td>0.79–1.70</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.63</td>
<td>0.50–0.80</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.01</td>
<td>0.90–1.12</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.00</td>
<td>0.94–1.07</td>
</tr>
<tr>
<td>Platelet count (K/mm³)</td>
<td>0.99</td>
<td>0.99–0.99</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>Standardized liver volume (mL/m²)</td>
<td>0.99</td>
<td>0.99–1.00</td>
</tr>
<tr>
<td>Standardized spleen volume ≥112.6 mL/m²</td>
<td>1.92</td>
<td>1.36–2.69</td>
</tr>
<tr>
<td>Skeletal muscle index (cm²/m²)</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>Visceral adipose tissue index (cm²/m²)</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue index (cm²/m²)</td>
<td>0.99</td>
<td>0.99–1.00</td>
</tr>
<tr>
<td>APRI</td>
<td>1.00</td>
<td>0.97–1.03</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>REACH-B</td>
<td>1.26</td>
<td>1.20–1.33</td>
</tr>
<tr>
<td>MELD</td>
<td>1.07</td>
<td>1.02–1.12</td>
</tr>
</tbody>
</table>

CHB, chronic hepatitis B viral infection; HCC, hepatocellular carcinoma; CI, confidence interval; HBeAg, hepatitis B e antigen; PT-INR, prothrombin time international normalized ratio; AFP, alpha fetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4 index; REACH-B, risk estimate for hepatocellular carcinoma in chronic hepatitis B; MELD, model for end-stage liver disease. ¹P<0.05.
Regarding the VAT index, the optimal cut-off value was set at 22.65 cm²/m² to predict hepatic decompensation. The estimated 1-, 5-, and 10-year cumulative incidences of hepatic decompensation in 990 patients with a VAT index <22.65 cm²/m² were 0.5%, 3.3%, and 6.1%, respectively, which were significantly higher than those of 1,179 patients with a VAT index ≥22.65 cm²/m² which were 0.8%, 2.4%, and 4.2%, respectively (HR=0.57, 95% CI=0.38–0.84, P=0.005) (Fig. 3C).

Figure 3. Kaplan–Meier estimation of cumulative incidences of HCC, hepatic decompensation, development of DM, and overall survival in patients stratified according to body composition measurements and organ volumes. (A) The cumulative incidence of HCC of 855 patients with a standardized spleen volume ≥112.6 mL/m² was significantly higher than that of 1,478 patients with a standardized spleen volume <112.6 mL/m² (P=0.001). (B) The cumulative incidence of hepatic decompensation of 367 patients with a standardized spleen volume ≥145.74 mL/m² was significantly higher than that of 1,040 patients with a standardized spleen volume <145.74 mL/m² (P=0.001). (C) The cumulative incidence of hepatic decompensation of 1,040 patients with a VAT index <22.65 cm²/m² was significantly higher than that of 1,293 patients with a VAT index ≥22.65 cm²/m² (P=0.012). (D) The cumulative incidence of DM of 1,680 patients with a VAT index ≥28.28 cm²/m² was significantly higher than that of 489 patients with a VAT index <28.28 cm²/m² (P=0.001). (E) The cumulative incidence of DM development of 1,249 patients with a standardized spleen volume ≥92.21 mL/m² was significantly higher than that of 920 patients with a standardized spleen volume <92.21 mL/m² (P=0.040). (F) The overall survival of 1,453 patients with a SAT index ≥39.08 cm²/m² was significantly higher than that of 716 patients with a SAT index <39.08 cm²/m² (P=0.001). HCC, hepatocellular carcinoma; VAT, visceral adipose tissue; DM, diabetes mellitus; SAT, subcutaneous adipose tissue.
Figure 3. Continued.

Table 3. The predictors for development of decompensation in 2,333 patients with compensated chronic liver disease from CHB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>P-value</td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>1.09</td>
<td>0.75–1.60</td>
<td>0.647</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (per 1 year)</td>
<td>1.06</td>
<td>1.04–1.08</td>
<td>&lt;0.001</td>
<td>1.06</td>
<td>1.04–1.08</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>1.01</td>
<td>0.98–1.05</td>
<td>0.493</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiviral therapy (yes)</td>
<td>1.14</td>
<td>0.79–1.66</td>
<td>0.488</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBeAg (positive)</td>
<td>1.21</td>
<td>0.78–1.88</td>
<td>0.397</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.369</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.52</td>
<td>0.42–0.64</td>
<td>&lt;0.001</td>
<td>0.59</td>
<td>0.45–0.77</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.91</td>
<td>0.69–1.19</td>
<td>0.469</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.00</td>
<td>0.91–1.10</td>
<td>0.967</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count (K/mm³)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.245</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized liver volume (mL/m²)</td>
<td>1.00</td>
<td>0.99–1.00</td>
<td>0.383</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized spleen volume ≥145.7 mL/m²</td>
<td>2.14</td>
<td>1.39–3.27</td>
<td>&lt;0.001</td>
<td>2.34</td>
<td>1.51–3.63</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Skeletal muscle index (cm³/m²)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.780</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral adipose tissue index ≥22.65 cm³/m²</td>
<td>0.61</td>
<td>0.41–0.90</td>
<td>0.013</td>
<td>0.57</td>
<td>0.38–0.84</td>
<td>0.005†</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue index (cm³/m²)</td>
<td>0.99</td>
<td>0.99–1.00</td>
<td>0.120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APRI</td>
<td>0.99</td>
<td>0.92–1.05</td>
<td>0.660</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.00</td>
<td>0.98–1.01</td>
<td>0.855</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REACH-B</td>
<td>1.14</td>
<td>1.07–1.21</td>
<td>&lt;0.001</td>
<td>1.05</td>
<td>0.98–1.12</td>
<td>0.144</td>
</tr>
<tr>
<td>MELD</td>
<td>1.07</td>
<td>1.02–1.12</td>
<td>0.008</td>
<td>1.02</td>
<td>0.96–1.07</td>
<td>0.564</td>
</tr>
</tbody>
</table>

CHB, chronic hepatitis B viral infection; CI, confidence interval; HBeAg, hepatitis B e antigen; PT-INR, prothrombin time international normalized ratio; AFP, alpha fetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4 index; REACH-B, risk estimate for hepatocellular carcinoma in chronic hepatitis B; MELD, model for end-stage liver disease.

†P<0.05.
Predictive factors for the development of DM

We excluded 164 patients who had DM at the time of enrollment to examine the development of DM. During the follow-up period, DM developed in 432 patients (19.9%, 432/2,169). The estimated 1-, 5-, and 10-year cumulative incidences for the development of DM were 2.7%, 10.7%, and 22.7%, respectively. Multivariate analysis revealed that VAT index (HR=1.01, 95% CI=1.01–1.01, \( P=0.001 \)) and standardized spleen volume (HR=1.01, 95% CI=1.01–1.01, \( P=0.001 \)) were among the significant predictors for the development of DM (Table 4), along with sex, age, and albumin level. The optimal cut-off value of the VAT index was set at 28.28 cm\(^2\)/m\(^2\) to predict the development of DM. The estimated 1-, 5-, and 10-year cumulative incidences of DM development in 1,680 patients with a VAT index ≥28.28 cm\(^2\)/m\(^2\) were 2.7%, 11.2%, and 24.8%, respectively, and were significantly higher than those of 489 patients with a VAT index <28.28 cm\(^2\)/m\(^2\) which were 2.5%, 9.0%, and 15.4%, respectively (HR=1.34, 95% CI=1.10–1.67, \( P=0.004 \)) (Fig. 3D).

Predictive factors for OS

We excluded 164 patients who had DM at the time of enrollment to examine OS. During the follow-up period, 120 patients (5.5%, 120/2,169) died. The causes of death were: unknown (n=104, 86.7%), liver cirrhosis-related complications (n=6, 5.0%), pneumonia (n=6, 5.0%), and progression of HCC (n=4, 3.3%). The estimated 1-, 5-, and 10-year OS were

### Table 4. The predictors for development of diabetes mellitus in 2,169 patients with compensated chronic liver disease from CHB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate</th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>Sex (female)</td>
<td>0.70</td>
<td>0.57–0.85</td>
<td>&lt;0.001</td>
<td>0.73</td>
<td>0.56–0.95</td>
<td>0.017^</td>
</tr>
<tr>
<td>Age (per 1 year)</td>
<td>1.04</td>
<td>1.03–1.05</td>
<td>&lt;0.001</td>
<td>1.05</td>
<td>1.04–1.06</td>
<td>&lt;0.001^</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>1.01</td>
<td>0.99–1.03</td>
<td>0.211</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiviral therapy (yes)</td>
<td>1.14</td>
<td>0.94–1.37</td>
<td>0.189</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.604</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.80</td>
<td>0.67–0.95</td>
<td>0.013</td>
<td>0.79</td>
<td>0.65–0.95</td>
<td>0.014^</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.98</td>
<td>0.91–1.07</td>
<td>0.690</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT-INR</td>
<td>0.99</td>
<td>0.93–1.06</td>
<td>0.820</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count (K/mm(^3))</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.116</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.431</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized liver volume (mL/m(^2))</td>
<td>1.01</td>
<td>1.01–1.01</td>
<td>0.004</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.075</td>
</tr>
<tr>
<td>Standardized spleen volume ≥92.21 mL/m(^2)</td>
<td>1.23</td>
<td>1.01–1.49</td>
<td>0.040</td>
<td>1.35</td>
<td>1.10–1.67</td>
<td>0.004^</td>
</tr>
<tr>
<td>Skeletal muscle index (cm(^3)/m(^2))</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.686</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral adipose tissue index ≥28.28 cm(^3)/m(^2)</td>
<td>1.56</td>
<td>1.21–2.02</td>
<td>0.001</td>
<td>1.34</td>
<td>1.13–1.74</td>
<td>0.001^</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue index (cm(^3)/m(^2))</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.927</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APRI</td>
<td>1.00</td>
<td>0.98–1.02</td>
<td>0.932</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIB-4</td>
<td>0.99</td>
<td>0.99–1.01</td>
<td>0.571</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELD</td>
<td>1.05</td>
<td>1.01–1.09</td>
<td>0.007</td>
<td>1.03</td>
<td>0.99–1.07</td>
<td>0.176</td>
</tr>
</tbody>
</table>

CHB, chronic hepatitis B viral infection; CI, confidence interval; PT-INR, prothrombin time international normalized ratio; AFP, alpha fetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4 index; MELD, model for end-stage liver disease. ^P<0.05.
99.6%, 98.1%, and 94.5%, respectively. Multivariate analysis revealed that the SAT index (HR=0.99, 95% CI=0.98–0.99, \(P=0.004\)) was significantly associated with OS, along with age, albumin, and the Model for End-Stage Liver Disease score (Table 5). The optimal cut-off value of the SAT index was set at 39.08 cm\(^2\)/m\(^2\) to predict OS. The estimated 1-, 5-, and 10-year OS in 1,453 patients with a SAT index \(\geq 39.08\) cm\(^2\)/m\(^2\) were 99.7%, 98.5%, and 95.6%, respectively, and were significantly higher than those of 716 patients with a SAT index <39.08 cm\(^2\)/m\(^2\) which were 99.4%, 97.2%, and 92.5%, respectively (HR=0.49, 95% CI=0.34–0.70, \(P<0.001\)) (Fig. 3F).

**DISCUSSION**

In this study, body composition data and spleen and liver volumes were automatically acquired from portal venous phase CT images using CNN in all patients. Standardized spleen volume was significantly associated with the development of HCC, hepatic decompensation, and DM; furthermore, the VAT index was correlated with the development of hepatic decompensation and DM. In addition, a higher SAT index correlated with increased OS.

Although ultrasound is currently the recommended modality for HCC screening in patients with CHB,\(^{23,24}\) its sensitivity for detecting HCC can be commonly reduced in situations such as limited sonic window, inherent liver blind spots, or coarse liver parenchyma masking focal lesions.\(^{25}\) Considering that CT scans can be performed as an alternative imaging modality in such circumstances,\(^{25}\) we expect that body composition and spleen volumetric data automatically and additionally obtained by applying CNN-based fully automated algorithm on CT data, can be used for opportunistically predicting the comprehensive prognosis of patients with CHB. Our study results are in line with those from previous

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>0.68</td>
<td>0.46–1.01</td>
</tr>
<tr>
<td>Age (per 1 year)</td>
<td>1.09</td>
<td>1.07–1.12</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>0.97</td>
<td>0.94–0.99</td>
</tr>
<tr>
<td>Antiviral therapy (yes)</td>
<td>1.00</td>
<td>0.70–1.43</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>1.00</td>
<td>1.00–1.01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.48</td>
<td>0.40–0.58</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.06</td>
<td>0.99–1.15</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.00</td>
<td>0.93–1.08</td>
</tr>
<tr>
<td>Platelet count (K/mm(^3))</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>1.00</td>
<td>1.00–1.01</td>
</tr>
<tr>
<td>Standardized liver volume (mL/m(^2))</td>
<td>1.00</td>
<td>0.99–1.00</td>
</tr>
<tr>
<td>Standardized spleen volume</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>Skeletal muscle index (cm(^2)/m(^2))</td>
<td>0.99</td>
<td>0.98–1.01</td>
</tr>
<tr>
<td>Visceral adipose tissue index (cm(^2)/m(^2))</td>
<td>0.99</td>
<td>0.99–1.00</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue index (\geq 39.08) cm(^2)/m(^2)</td>
<td>0.46</td>
<td>0.32–0.66</td>
</tr>
<tr>
<td>APRI</td>
<td>1.01</td>
<td>0.99–1.03</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.00</td>
<td>0.99–1.01</td>
</tr>
<tr>
<td>MELD</td>
<td>1.12</td>
<td>1.08–1.17</td>
</tr>
</tbody>
</table>

CHB, chronic hepatitis B viral infection; CI, confidence interval; PT-INR, prothrombin time international normalized ratio; AFP, alpha fetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis-4 index; MELD, model for end-stage liver disease.

\(^1\)\(P<0.05\).
Our study results showed the prognostic role of spleen volume in predicting the development of HCC and hepatic decompensation. As liver fibrosis and cirrhosis advance, the spleen tends to enlarge owing to both the accumulation of portal flow and tissue hyperplasia characterized by a combination of angiogenesis, fibrogenesis, enlargement, and hyperactivation of the splenic lymphoid compartment. Therefore, spleen enlargement was reported as a potential surrogate marker of the severity of liver fibrosis and portal hypertension and showed a significant association with the development of HCC and hepatic decompensation in previous studies, which aligns with our results. However, these previous studies determined a spleen enlargement of more than 12 cm on ultrasonography or acquired splenic volume by applying a semi-automated software to CT scans. In contrast, in this study, we obtained spleen volume using a deep learning-based fully automated segmentation tool, saving researchers’ time and efforts.

The association between CHB and DM has been controversial, and some previous studies reported the increased prevalence of DM in patients with CHB, while others did not. In our study, the spleen volume was a significant predictor of DM. Several mechanisms have been suggested to explain the association between hepatitis B viral (HBV) infection and the prevalence of DM. The liver’s impairment resulting from HBV infection can potentially lead to disruptions in glycometabolism, owing to its crucial role in maintaining glucose homeostasis by managing both glucose storage and release. Therefore, the significant correlation between the spleen volume and DM in our study may be attributable to the association between DM and HBV infection-induced chronic inflammation of the liver, which was represented by increased splenic volume.

In our study, a higher VAT index was associated with hepatic decompensation and DM, whereas a higher SAT index was significantly correlated with increased OS. In previous studies, obesity, represented by the body mass index, was a significant risk factor for decompensation in patients with cirrhosis of all etiologies. Moreover, obesity-related changes in insulin and leptin may change intrahepatic vascular resistance, leading to portal hypertension. However, body mass index alone may not be an accurate predictor of outcomes since it does not reflect the distribution of fat deposition. Recently, growing evidence indicates that adipose tissue plays an active role in influencing endocrine function, immunity (i.e., leptin and adiponectin), and angiogenesis (i.e., angiopoietin-2, vascular endothelial growth factor, leptin, and adiponectin). Adipose tissue is currently divided into SAT and VAT based on location and functions. The VAT index was an independent predictor of DM in our study, which can be explained by previous studies showing significant correlations between increased visceral adiposity with insulin resistance in patients with DM. In contrast, the favorable effects of increased SAT on survival were demonstrated in patients with various oncologic diseases and cirrhosis, which are concordant with our study results. The reasons for the protective effects of high subcutaneous adiposity were not clearly identified; however, potential explanations include the association between leptin produced by SAT and better insulin sensitivity and energy metabolism, as well as less prevalent cachectic state inducing energy exhaustion in patients with increased SAT. Based on these results from ours, fully automated body composition measurements using CNN on CT data which quantifies the distribution of fat deposition, rather than merely determining the presence of obesity according to the body mass index, may provide more accurate information regarding prognosis.

Our study has several limitations. First, this was a retrospective study from a single tertiary referral center; therefore, selection bias was unavoidable. Furthermore, our study results were not externally validated in other patient cohorts. Therefore, further studies are warranted to validate our study results. Second, our study did not provide longitudinal follow-up data which might have shown different results.

In conclusion, a larger spleen volume was significantly associated with HCC, hepatic decompensation, and DM occurrence. A higher abdominal VAT index was correlated with the development of DM, while a higher SAT index was correlated with increased OS in patients with CHB. Deep learning-based automatically measured spleen volume, abdominal VAT and SAT indices may be used as opportunistic prognostic factors in patients with CHB.

Authors’ contribution

Jeongin Yoo: analysis and interpretation of data; drafting manuscript; critical revision of the manuscript; obtained funding. Heejin Cho: acquisition of data; critical revision of
the manuscript. Dong Ho Lee: study concept and design; analysis and interpretation of data; supervision; critical revision of the manuscript. Eun Ju Cho: study concept and design; supervision; critical revision of the manuscript. Ijin Joo: study concept and design; acquisition of data. Sun Kyung Jeon: study concept and design; acquisition of data. All authors approved the final version of the manuscript.

Acknowledgements
This study was supported by a research grant (No. 04-2021-2310) from Seoul National University Hospital, Seoul, Korea.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

20. Korean Liver Cancer Association (KLCA) and National Cancer


Letter to the Editor

Letter regarding “Evidence-based hyponatremia management in liver disease”

Daphne J. Theodorou¹, Stavroula J. Theodorou², and Ioannis V. Mitselos³

¹Department of Radiology, General Hospital of Ioannina; ²Department of Radiology, University Hospital of Ioannina; ³Department of Internal Medicine, Gastroentology Unit, General Hospital of Ioannina, Ioannina, Greece

Keywords: Osmolar derangement; MRI; Brain; Edema; Myelinolysis.

Dear Editor,

Recently, Ryu et al.¹ provided an extensive review of hyponatremia, the most common electrolyte abnormality in hospitalized patients, focusing on patients with liver disease. Indeed, this serious disorder of osmolality continues to take a huge toll on patients with hepatic disease, accounting for complications that are associated with high morbidity or death.²,³ As such, the authors thoroughly examined the pathophysiology, laboratory findings and clinical aspects of hyponatremia to formulate guidelines for the effective management of patients with advanced liver disease.

Because hyponatremia is multifactorial and complex, the clinical diagnosis and management of adverse effects of osmolar derangement are difficult, particularly in critically ill patients. Perhaps just as important would be drafting a plan for the correction of hyponatremia without complication of myelinolysis, in each individual case.⁴ Indeed, patients with advanced liver disease are at high risk for developing grave neurological complications including hepatic encephalopathy, and osmotic demyelination syndrome (ODS) secondary to marked disturbances of electrolytes. The clinical course of ODS is biphasic: first, it begins with hyponatremic encephalopathy that appears to improve transiently following rapid elevation of serum sodium levels; and, second, 2–3 days after the correction or overcorrection of sodium, ensuing myelinolysis can cause spastic quadriplegia, pseudobulbar palsy, coma or death.⁵ Although the authors are indeed correct that ODS is a rare and life-threatening neurologic complication resulting from excessive hyponatremia therapy, neuronal changes may be reversible and the evolution of encephalopathy can ideally be monitored.

Advances in magnetic resonance (MR) imaging of the brain have revolutionized the understanding of complex diagnoses of many neurological disease processes. In ODS, myelinolysis, the dominant feature of this particular form of toxic encephalopathy, affects primarily the white matter causing loss of myelin with relative sparing of the neurons. Pathological changes of ODS include destruction of the myelinated nerve sheaths and loss of oligodendrocytes, which are most susceptible to osmotic disturbances. These structural neuronal abnormalities are foremost reflected on the distribution of the MR imaging changes of osmotic myelinolysis in the white matter, in pontine or extrapontine sites, which as

Corresponding author : Daphne J. Theodorou
Department of Radiology, General Hospital of Ioannina, 13 Papadopoulos street, Ioannina, Greece
Tel: +302651038003, Fax: +302651038003, E-mail: daphne_theodorou@hotmail.com
https://orcid.org/0000-0002-2477-3871

Editor: Hyo Jung Cho, Ajou University School of Medicine and Graduate School of Medicine, Korea Received : Jun. 14, 2023 / Revised : Jun. 16, 2023 / Accepted : Jun. 21, 2023
said parallels the distribution of oligodendrocytes in the brain.\textsuperscript{5,6} MR imaging reportedly has played a key role in the determination of both the presence and extent of myelinolysis related to osmotic dysregulation.\textsuperscript{7,8} The well-recognized addition of diffusion-weighted imaging (DWI), a specialized imaging technique, to conventional MR sequences allows for early detection of changes in diffusion of the water molecules reflecting cytotoxic brain edema, caused by hyponatremia (Fig. 1). For example, in a previous study Ruzek et al.\textsuperscript{5} were able to identify myelinolysis within 24 hours of onset of tetraplegia on DWI. On MR images, ODS lesions may display decreased signal intensity on T1-weighted images, frequently with a symmetric distribution. On the T2-weighted and fluid-attenuated inversion recovery (FLAIR) images, osmotic demyelination lesions show increased signal intensity with corresponding high signal intensity on DWI (and low ADC values), indicating restricted diffusion due to active cytotoxic edema in the affected brain areas.\textsuperscript{5,8,9} With normalization of intracellular hypotonicity and re-establishment of equilibrium of osmolality, gradual improvement of the brain lesions may be seen on the MR images. Further, recent evidence indicates that oligodendrocytes that survive demyelination can remyelinate.\textsuperscript{10} As such, the regenerative potential of neuroglial cells may need to be clinically addressed or monitored on the imaging studies for prognostication purposes, in those patients with liver disease and resolving ODS. Hyponatremia and attempted overly rapid correction of the electrolyte imbalance may cause extreme osmotic stress that can be damaging to the brain, with debilitating neurologic sequelae. DWI can detect changes of water diffusion in the brain and is useful in diagnosis of cytotoxic edema associated with ODS. In any cases, the authors would agree that MR imaging is a powerful tool that needs to be added to the diagnostic armamentarium of clinicians managing hyponatremia and its neurological complications, in patients with liver disease.

**Authors’ contributions**

The authors contributed equally to conceptualization, analysis and writing of this manuscript.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

1. Ryu JY, Baek SH, Kim S. Evidence-based hyponatremia manage-
Dear Editor,

We recently read the review article by Ko et al. titled, “Risk factors in nonalcoholic fatty liver disease”. The article has in depth explained the various risk factors associated with non-alcoholic fatty liver disease (NAFLD) and also highlighted NAFLD associated complications having an increased risk of morbidity and mortality from cardiovascular disease (CVD) and malignancy. The review has also emphasized on increasing trend of prevalence and mortality rate due to NAFLD and the risk factors driving the change.

One of the risk factors mentioned is diet, and in it the authors have mentioned how fructose promotes lipogenesis and impairs oxidation of lipids and produces oxidative stress, all of which contribute to NAFLD. Here I would like to emphasize that fructose is consumed mainly as added sugar in form of sucrose, fruit juices, sugar sweetened beverages etc. As per the World Health Organization (WHO) recommendations, there is an acceptable limit to added sugars for beverages without causing any harm to the body. According to these guidelines, usually upto 10% of the total calorie requirement or less than 25 g/day or 6 tsp of added sugar or 1 sweetened beverage / week has been recommended.2,3 Here, I would like to highlight the need to circulate the message that fructose needs to be consumed within permissible limit to avoid health hazards including NAFLD.

Authors’ contribution
Drafting of the article: Abhijit Pratap, Pradnya Phalak. Critical revision: Umesh More, Anita Deshmukh.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
1. Ko E, Yoon EL, Jun DW. Risk factors in nonalcoholic fatty liver disease. Clinical and Molecular Hepatology 2023;29:1046-1047

Corresponding author: Abhijit Pratap
Dr D Y Patil Medical College Hospital and Research Centre, Pimpri, Pune - 411018, Maharashtra, India
Tel: +912027805000, Fax: +912027420010, E-mail: abhijit.pratap@dpu.edu.in
https://orcid.org/0000-0001-8265-7687

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

Received: Jul. 24, 2023 / Revised: Aug. 1, 2023 / Accepted: Aug. 1, 2023

Abbreviations:
NAFLD, non-alcoholic fatty liver disease; CVD, cardiovascular disease


Dear Editor,

We appreciate Theodorou and coauthors for introducing clinical manifestation of osmotic demyelination syndrome (ODS) including reversible and irreversible sequelae and emphasizing that magnetic resonance (MR) imaging is needed for early detection and proper diagnosis of ODS in hyponatremic patients with liver disease. Furthermore, MR imaging might have prognostic value due to the regenerative potential of neuroglial cells in patients with liver disease and resolving ODS. Generally, ODS is diagnosed clinically and by MR image.

Our review focused on pathophysiology, diagnosis, and treatment of hyponatremia in patients with liver diseases. We were unable to discuss ODS itself thoroughly. ODS is symmetric, non-inflammatory demyelination of neurons, which can be classified into two types based on location: central pontine myelinolysis and extrapontine myelinolysis. It occurs as a result of apoptosis of oligodendrocytes and infiltration of myelin degrading macrophages. Hyponatremia and overly rapid correction of hyponatremia have been well-known as potent causative factors of ODS. The only recommendation of ODS till date is conservative treatment. The best approach is focused on prevention strategies with two aspects: identifying patients at risk and implementing proper correction, especially with a strict maximum of 8 mmol/L per day for individuals at risk of ODS. However, it should be noted that ODS can occur even in the absence of hyponatremia or overcorrection of hyponatremia in patients with high risk of ODS. Patients with chronic alcohol consumption (the most common) or liver cirrhosis/liver transplantation (third largest group) are more susceptible to ODS because of reduced ability of astrocytes to synthesize new intracellular osmolytes in response to osmotic changes. In a recent study involving 547,544 adult inpatients with cirrhosis, ODS was found to be developed in only 0.02% of patients. It was associated with alcohol-related cirrhosis, young age, and female gender. ODS was not associated with liver disease severity (decompensated cirrhosis) or specific complications including ascites or hepatic encephalopathy. Patients undergoing liver transplant are also at risk for rapid correction of serum sodium due to intraoperative administration of ...
intravenous crystalloids, blood products, and sodium bicarbonate during operation, in addition to preexisting conditions.\textsuperscript{4} The incidence of ODS is 0.8% to 1.4%.\textsuperscript{3,4} Symptom onset is known to be within 1 to 2 weeks after liver transplantation.\textsuperscript{3} Additionally, although relatively less prevalent, ODS can occur in patients with burns, malnutrition, chemotherapy, diabetes mellitus, adrenal insufficiency, acquired immune deficiency syndrome, severe illness/sepsis, hypoglycemia/hypokalemia/hypophosphatemia, and renal disease with or without liver disease.\textsuperscript{4}

In summary, individuals with advanced liver disease are more susceptible to ODS. For patients with liver diseases accompanied by aforementioned predisposing disease or circumstances or those undergoing liver transplantation, greater attention should be paid to ODS.

**Authors’ contribution**

JYR: drafting of the manuscript. SHB, SK: critical review and final approval of the manuscript.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


**Abbreviations:**

ODS, osmotic demyelination syndrome; MR, magnetic resonance
Dear Editor,

Thank you for your interest in our paper.\textsuperscript{1,2} As highlighted, the association of added fructose and sugar-sweetened beverages with non-alcoholic fatty liver disease (NAFLD) is well-established.\textsuperscript{3} The National Health and Nutrition Examination Survey (NHANES) data have demonstrated a link between increased added sugar consumption and a higher prevalence of NAFLD and obesity.\textsuperscript{4} Numerous epidemiological studies have also indicated a dose-response relationship between sugar-sweetened beverage consumption and both NAFLD prevalence and incidence.\textsuperscript{5} Consumption of fructose causes harmful changes to the composition of the gut microbiome.\textsuperscript{6}

The Framingham Heart Study reconfirmed that frequent consumers of sweetened beverages face a significantly elevated risk of developing NAFLD compared to non-consumers.\textsuperscript{7} Data from experimental studies have also consistently aligned with these findings. Notably, research involving the administration of sugar-sweetened beverages to healthy subjects resulted in increased liver fat over time measured by magnetic resonance spectroscopy.\textsuperscript{8} Conversely, studies restricting added fructose intake in children with high baseline fructose consumption demonstrated a reduction in liver fat on magnetic resonance spectroscopy. Both experimental and clinical studies have suggested an association between fructose intake and NAFLD. There is a clear association between fructose intake of added sugars and increases in obesity and NAFLD. Therefore, it is recommended to reduce the amount of refined carbohydrates, especially fructose in patients with NAFLD.\textsuperscript{9}

However, the results of studies on the effect of fructose beyond added sugars, including fructose contained in fruit, on the development of NAFLD have been more diverse. The role of fructose in fruits and added fructose (industrial corn syrup) is thought to be very different. Epidemiological data from Finland revealed an intriguing inverse relationship between fructose intake from fruits and NAFLD.\textsuperscript{10} This contradictory observation might be attributed to higher levels of fruit consumption and the potential mitigating components against fructose’s adverse effects. Although fruit also contains fructose, it is less likely to cause metabolic syndrome because it is low in fructose and contains components that can combat...
the effects of fructose. In the Rotterdam Study of 3,882 participants, monosaccharide and disaccharide intake showed an inverse correlation with NAFLD prevalence, but this effect disappeared after adjusting for metabolic covariates and body mass index. Considering the complexity of this relationship, recent systematic reviews have underscored the insufficiency of data on the overall impact of fructose, including natural sources, on NAFLD.11

Authors’ contribution

Acknowledgements
This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023-00217123).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
7. Park WY, Yiannakou I, Petersen JM, Hoffmann U, Ma J, Long MT. Sugar-sweetened beverage, diet soda, and nonalcoholic fatty liver disease over 6 years: The frameingham heart study. Clin Gastroenterol Hepatol 2022;20:2524-2532.e2.

Abbreviations:
NAFLD, nonalcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey
The role of the hepatic autonomic nervous system

Qiankun Luo*, Pan Liu*, Yifei Dong*, and Tao Qin

Department of Hepatobiliary and Pancreatic Surgery, Zhengzhou University People’s Hospital, Henan Provincial People’s Hospital, Zhengzhou, China

Corresponding author: Tao Qin
Department of Hepatobiliary and Pancreatic Surgery, Zhengzhou University People’s Hospital, Henan Provincial People’s Hospital, No. 7, Weiwu Road, Jinshui District, Zhengzhou, Henan 450003, China
Tel: +86-0371-65580365, Fax: +86-0371-65951056, E-mail: qtgoodfreecn@zzu.edu.cn
https://orcid.org/0000-0001-8791-3555

*These authors contributed equally to this work.
The regulation of liver function and homeostasis are well-known to be controlled by both the sympathetic and parasympathetic nervous systems. Growing research has emphasized the significance of the autonomic nervous system in liver diseases. However, the mechanisms underlying these processes are still not fully understood.

Preganglionic sympathetic innervation of the liver originates from the T7–T12 thoracic nerves, while the postganglionic sympathetic innervation from visceral nerves originates from the celiac and superior mesenteric ganglia. Parasympathetic innervation (vagus nerve) stems from the medulla oblongata. The anterior plexus forms a sheath around the hepatic artery and enters the liver via the hepatic artery. The posterior plexus is predominantly distributed along the extrahepatic bile ducts and portal vein, with branches communicating with those from the anterior plexus. The neural plexuses in the liver closely interact with liver sinusoidal cells, hepatic stellate cells (HSCs), Kupffer cells (KCs), and hepatic progenitor cells (HPC).

In various models of chemical liver injury, the sympathetic nervous system (SNS) contributes to liver inflammation, injury, and fibrosis. Conversely, vagal nerve signals promote liver regeneration by inhibiting apoptosis and exhibiting anti-inflammatory effects.

SYMPATHETIC NERVOUS SYSTEM

The SNS contributes to the development of non-alcoholic fatty liver disease and hepatocellular carcinoma (Table 1). In liver injury models, increased expression of alpha and beta-adrenergic receptors (α/β-AR) and neuropeptide Y (NPY) receptors on HSCs promotes liver fibrosis and cirrhosis by inducing HSCs proliferation and secretion of inflammatory factors. Epinephrine (EPI) and norepinephrine (NE) induce the production of collagen-1α2 (COL1A2), transforming growth factor-beta (TGF-β), and induce the activation of NF-κB by acting on HSCs. Moreover, the SNS promotes liver inflammatory microenvironment formation and hepatocellular carcinoma development by regulation of interleukin (IL)-6 and TGF-β through activation of α1-AR on KCs. Soeda et al. showed that the SNS inhibits HPC and promotes liver injury by activating α1-AR. However, β-AR agonist isoproterenol-mediated β-AR activation rescues acetaminophen (APAP)-induced liver injury by promoting HPC proliferation through Wnt signaling activation.

PARASYMPATHETIC NERVOUS SYSTEM (PNS)

In contrast to the SNS, vagal signaling exerts protective effects on hepatic damage (Table 1). In a mouse model, the cholinesterase inhibitor neostigmine reduced APAP-induced acute liver failure and improved the survival rate of the mice. The α7 nicotinic acetylcholine receptor (α7AChR) agonist exhibits an anti-apoptotic effect on Fas-induced hepatitis by suppressing KC-generated reactive oxygen species. Morever, parasympathetic nerve-secreted acetylcholine (Ach) inhibits expression of tumor necrosis factor (TNF) and IL-6 through activation of Src kinase by acting on the α7AChR of KCs, thus suppressing lipopolysaccharide-induced hepatitis. Muscarinic acetylcholine receptor type 3 (M3 AChR) is proven to be expressed on the surfaces of cholangiocytes and HPC. Activation of M3 AChR plays an important role in cholangiocyte and HPC proliferation, anti-apoptosis, and liver regeneration. Furthermore, PNS activation increases the expression of IL-6 in macrophages by acting on muscarinic Ach

**Abbreviations:**

HSCs, hepatic stellate cells; KCs, Kupffer cells; HPC, hepatic progenitor cells; SNS, sympathetic nervous system; α/β-AR, alpha and beta-adrenergic receptors; NPY, neuropeptide Y; EPI, epinephrine; NE, norepinephrine; COL1A2, collagen-1α2; TGF–β, transforming growth factor-beta; IL, interleukin; APAP, acetaminophen; PNS, parasympathetic nervous system; α7AChR, α7 nicotinic acetylcholine receptor; TNF, tumor necrosis factor; M3 AChR, muscarinic acetylcholine receptor type 3; mAChRs, muscarinic Ach receptors

**Keywords:** Sympathetic nervous system; Parasympathetic nervous system; Adrenergic receptor; Acetylcholine receptor; Hepatic diseases

receptors (mACHRs). Furthermore, IL-6 promotes hepatic FoxM1 activation that stimulates hepatocyte proliferation through signal transducers and activators of transcription. Therefore, parasympathetic signaling may be a potential therapeutic target for patients with liver injury, hepatitis, and hepatic tumors.

However, studies showed that NE promoted hepatocellular carcinoma metastasis through α1/β2-AR activation, while the vagus nerve had a suppressive role in liver metastasis in mice. Moreover, in a mouse model of Fas-induced fulminant hepatitis, the release of NE by the SNS demonstrates an anti-apoptotic and hepatoprotective effect by up-regulating the expression of IL-6. Notably, these studies were carried out in animal models, and many of these studies employed systemic administration of chemical sympathectomy, which does not selectively affect the liver. Therefore, further investigation is needed to clarify the role of the autonomic nervous system in liver.

Author’s contribution

Acknowledgements
This work was supported by the National Natural Science Foundation of China (31671440).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Instructions for Authors

General Information

The Clinical and Molecular Hepatology publishes original basic and clinical research on liver diseases. Manuscripts should be submitted electronically (https://mc04.manuscriptcentral.com/cmh). The journal is published in English on 1st in January, April, July, and October. Authors lacking ability with English syntax should seek the appropriate editorial assistance prior to submitting their manuscripts. These guidelines are in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals,” published by the International Committee of Medical Journal Editors at http://www.icmje.org.

The Editorial Office, the Clinical and Molecular Hepatology, Room A1210, Mapo Trapalace, 53 Mapo-daero, Mapo-gu, 04158, Seoul, Korea
Tel.: 82-2-703-0051, Fax: 82-2-703-0071, E-mail: kasl@kams.or.kr

Types of Manuscripts

Contributions may be submitted as original articles, review articles, editorials and special topics. Special topics cover guidelines, meeting reports and hepatology issues elsewhere. Review articles, editorials and special topics are invited by the editorial board. However, authors who are interested in contributing reviews can submit reviews and are subjected to peer review. Letters to the editor may be subjected to peer review and undergo editing for clarity and brevity.

Ethical Conduct of the Study and the Report

All investigations involving human participants must be conducted according to the ethical guidelines of the Declaration of Helsinki, and be approved by the institutional review board. For studies involving animal experimentation, author(s) must provide assurance that all the animals received humane care according to the criteria outlined in the NIH “Guide for the Care and Use of Laboratory Animals”. The author must state that the use of animals (means all mammals and birds) in the manuscript was approved by the institutional Animal Ethical Committee (AEC) in accordance to the article 14th of Korean Animal Protection Law, or equivalent, in the paper. It must be clearly stated that animal use has complied to the article 13th of Korean Animal Protection Law (The principles of animal use) and the relevant institutional polices in the manuscript. Copies of the protocol approved by institutional AEC or equivalents, must be available for review by the editor if necessary.

The corresponding author must give written assurance that neither the submitted material nor portions thereof have been published previously or are under consideration for publication elsewhere. Any material that could constitute prior or concurrent publication of similar data by any one of the authors should be submitted with the manuscript. It is assumed that the corresponding author speaks for his or her co-authors and certifies that all the listed authors meaningfully participated in the study and that they have seen and approved the final manuscript.

Authors should acknowledge any commercial affiliation or consultancy that could be constructed as potential conflicts of interest under a heading “Conflict of Interest statement” prior to the references.

For the policies on the research and publication ethics not stated in this instructions, ‘Good Publication Practice Guidelines for Medical Journals (https://www.kamje.or.kr/board/view?b_name=bo_publication&bo_id=7&per_page=)’ or ‘Guidelines on good publication (http://www.publicationethics.org.uk/guidelines)’ can be applied.

Ensure correct use of the terms sex (when reporting biological factors) and gender (Identity, psychosocial or cultureral factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender.

If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases, (e.g., prostate cancer).

Authors should define how they determined race or ethnicity and justify their relevance.
Organization of the Manuscript

The manuscript should be written in A4 (21×30 cm) paper in double space texts by leaving 3 cm space in the right, left, top and bottom sides at 10 point fonts.

Original articles

Original articles describing clinical and basic studies in the field of hepatology. Manuscripts are expected to be well-organized and clearly written. They should not exceed 6,000 words, including the abstract, references, tables, and figure legends. No more than 8 figures and tables, with a maximum of 6 panels per figure. It is permitted for you to submit additional methodological details, non-essential figures or portions of your manuscript as supplementary material for online publication only. References cited in the main text may not be listed in the supplementary materials. The only references be listed in the supplement are those cited exclusively in the supplement. References should not exceed a maximum of 50.

Original article must arranged as follows: (1) title page (2) abstract (250 words or less with a list of 5 or less key words), (3) introduction, (4) materials and methods (or patients and methods), (5) results, (6) discussion, (7) acknowledgements, (8) conflict of interest statement (9) references, (10) tables, and (11) figure legends.

In case of submission of original articles (not applicable for reviews, editorials, and letters), authors should summarize the contents of the article in a concise, pictorial form designed to easily understand main findings of the work described in the article. Graphical abstracts should be submitted as a separate JPG or TIFF files at the online submission step of file upload. The submission of the graphical abstract is mandatory when submitting an original article. Graphical abstract should be provided as an image with a minimum size of 531 × 531 pixels (height × width) using a minimum resolution of 600 dpi. When submitting a larger image, please make sure to use the same ratio. Also, please note that your image will be scaled proportionally to fit in the available window, which is a rectangle with a size of 200 × 500 pixels.

Review articles

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

Editorials

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

Letters to the editor

Letters to the editor should be related to a recent article published in the Clinical and Molecular Hepatology within previous two years. Letters to the editor must arranged as follows: (1) title page, (2) body (3) references (maximum of 15), and (4) a maximum number of 1 tables or figures is allowed. The length of an letter to the editor should not exceed 800 words, and the maximum number of authors is 6. Abstract is not required.

Correspondence

The correspondence consists of replies on editorials from the authors of the original publication in the Clinical and Molecular Hepatology. The length of an correspondence should not exceed 1,500 words and 1 table or 1 figure is allowed. References should not exceed a maximum of 15. Correspondence letters are not usually peer reviewed, but we might invite replies from the authors of the original publication.

Special topics

Special topics should be no longer than 800 words with 10 or less references.
Snapshot

Snapshot consists of a large single page figure with schematic diagrams and tables that graphically summarize current knowledge about a particular subject within the field of hepatology. A detailed figure legend which includes all relevant information can be included and may be incorporated into the main figure. The figure is accompanied by a short summary article that should not exceed a maximum of 600 words. References should not exceed a maximum of 10. The snapshot should contain a descriptive title.

1. Title page
Provide a concise title. List the full names of all authors and their institutional affiliation. In a multi-authored work involving more than a single institution, indicate individual affiliation by means of superscript Arabic numbers. Indicate a change of address in a similar fashion. List the footnotes to the title page. Provide the contact information for the corresponding author (name, address, telephone number, fax number, e-mail address and Orcid ID), and running title (Less than 50 characters). All abbreviations should be explained in this page (e.g. AFP, alpha fetoprotein; ALT, alanine aminotransferase). The Clinical and Molecular Hepatology employs a system to screen plagiarism (CrossRef). When submitting your manuscript to this journal, you accept that your manuscript may be screened for plagiarism against previously published material.

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

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

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

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

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

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

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

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

Articles in journals

Literature in press
An online article that has not yet been published in an issue can be cited by its Digital Object Identifier (DOI). The DOI will remain valid and allow an article to be tracked even after its allocation to an issue.

Book chapters

Abstract or Article in a supplement

Websites

10. Permissions
Direct quotations, tables or illustrations taken from copy-righted material must be accompanied by written permission for their use from the publisher. The permission is presented as a footnote or addition to the legend and it must provide complete information as to the source. Photographs of identifiable persons must be accompanied by a signed release that indicates their informed consent.

11. Abbreviations
Please include an alphabetical list of all non-standard abbreviations used within the manuscript. Please do not abbreviate unless a term is used more than five times in a paper. In this case, the abbreviation should be spelled out, in its first use in the text with the abbreviated form in parentheses, and it should also be listed on the footnote page. Abbreviations used in figures or tables should be defined in the legend.
12. Drug names
Use generic names. The proprietary name may be mentioned in parenthesis. The names and locations (city and state or country) of manufacturers should be included in parentheses when mentioning proprietary drugs, tools, instruments, software, etc.

13. Tables
Prepare tables on individual sheets of paper, double spaced and numbered consecutively with Arabic numerals in the order of their appearance in the text. The title of tables should be written concisely in clauses and phrases. The first letter of the table title starts with a capital letter. Explain all abbreviations and symbols such as *, †, ‡, §, ¶, ‡‡, §§. Do not duplicate the material presented in a figure.

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

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

Peer Review and Publishing
The journal utilizes blind peer-review in evaluating manuscripts for publication. Submitted papers will be reviewed by at least two referees, and decisions will be available in approximately one months. With respect to the revision and resubmission of manuscripts, it is the journal’s policy to allow a couple of resubmission only, which should be received within 2 months from the time of receipt of the initial review letter. In general, a manuscript requiring more than a couple of revision or returned beyond 2 months will be handled as a new submission. The journal does not have article submission charges.

Article processing charge (APC)
As of January 1, 2022, the Clinical and Molecular Hepatology charges a publication fee of US$1,000 per accepted article. The authors will receive an invoice for APC shortly after the corrected proof of their accepted manuscript has been finalized. Please note that only “original articles” are subject to article processing charges.

Fast-track review (optional)
A fast-track review process is available for authors who desire quick publication of their papers. Fast-track manuscripts will be handled by the Editor in Chief, and the first decision following a full peer-review of the manuscript will be made within 7 days of submission. The accepted papers will be published within 2 weeks from the date of acceptance, in the next issue of the Clinical and Molecular Hepatology. An additional non-refundable processing fee (US$1,000) will be charged for the initiation of the fast-track process. A fast-track review does not guarantee acceptance. The journal is editorially independent and will assess your manuscript according to its own criteria. If your article is finally accepted, an article processing charge of US$1,000 will be additionally charged. If you wish to submit your article using the fast-track review process, please contact the Editorial Office in advance to arrange a peer-review process.

Cover page (optional)
For the authors who wish to publish their paper as a cover page article, we offer full support in producing the illustration to go on the cover. The Clinical and Molecular Hepatology charges US$1,000 for the cover page illustration work. If you are interested, please contact the Editorial Office.
Copyright Transfer

Copyright for all material published in *the Clinical and Molecular Hepatology* is vested in Korean Association for the Study of the Liver. In accordance with the Copyright Act, all manuscripts must be accompanied by a copyright transfer form signed by all authors and that follows these guidelines. Statements and opinions expressed in the articles and communications in *the Clinical and Molecular Hepatology* are those of the author(s) and do not necessarily reflect the opinions of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants or endorses any product or service advertised in the journal; nor do they guarantee any claim made by the manufacturer of such product or service.
Copyright Transfer and Conflict of Interest Disclosure Form

Manuscript No.  Date.
Manuscript Title.

Copyright Transfer Form

In consideration of editors and publisher’s effort in reviewing and editing our/my article, the undersigned authors hereby transfer, convey, and assign all copyrights in the article to Korean Association for the Study of the Liver (KASL). The copyright transfer covers the right to print, publish, distribute and sell throughout the world the said contribution and parts thereof, including all revisions or versions and future editions, in all forms and media.

The authors certify that I have participated in the intellectual content, the analysis of data, and the writing of the article, to take public responsibility for it. The authors reviewed the final version of the article, believe it represents valid work and approve it for publication.

The authors certify that none of the material in the manuscript has been published previously, is included in another manuscript. The authors also certify that the article has not been accepted for publication elsewhere, nor have they assigned any right or interest in the article to any third party. The authors will obtain and include with the manuscript written permission from any respective copyright owners for the use of any text, figures, and tables that have been previously published. The authors agree that it is their responsibility to pay fees charged for permissions.

Conflict of Interest Disclosure Form

The authors certify that I have reviewed conflict of interest form, defined by the International Committee of Medical Journal Editors (ICJME) found at the following URL: http://www.icmje.org/, and attached separate ICMJE Form for Disclosure of Potential Conflicts of Interest that might pose a conflict of interest in connection with the submitted article.

Author (Print)  Affiliation  Position  Signature

Position indicate current status at your affiliation; professor, fellow, resident, student, post doc.

The copyright transfer agreement and conflict of interest disclosure form should be signed and faxed or submitted by e-mail to the Editorial Office of the Clinical and Molecular Hepatology at Fax: 82-2-703-0071, E-mail: kasl@kams.or.kr. Manuscript can not be published until the completed form of copyright transfer form has received by the Editorial Office.
### The Clinical and Molecular Hepatology Submission Checklist

Please read this checklist carefully to ensure that your manuscript is complete and in compliance with the CMH Guide for Authors.

<table>
<thead>
<tr>
<th>1) General Format</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] Did you have the title page, abstract, the text (introduction, materials and methods, results, and discussion), acknowledgements, conflict of interest statement, references, tables, and legends for figures?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[2] Is the manuscript double-spaced in an A4-size paper?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[3] The manuscript of special topics should not be longer than 800 words.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[4] The number of authors for letters to the editor must not exceed 6.</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2) Abstract</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] Abstract must contain 250 words or less and must be organized as follows: Backgrounds/Aims, Methods, Results, and Conclusions.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[2] Five or less key words should be provided at the end of the abstract.</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3) Introduction, Methods, Results, Discussion, Acknowledgements, Conflict of Interest Statement, References</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] Identify the committee(s) approving the study protocol and include a statement of compliance with ethical regulations.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[2] An acknowledgement of persons who made a assistance and provided special reagents may be included. Grant and financial support related with the work should be specifically stated.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[3] Please state any conflicts of interest.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[4] All citations in the paper have a complete and accurate reference in the reference list. The number of references in special topics should be 10 or less.</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4) Tables and Figures</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] Prepare tables on individual sheets of paper, double spaced and numbered consecutively with Arabic numerals in the order of their appearance in the text.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[3] Figure legends should be typed consecutively on a separate sheet of paper.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[4] Figures should be supplied in the JPG or TIFF format at a final resolution of 600 dpi or higher.</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Protect from Various Liver Disease with Legalon®

As the original brand of silymarin, Legalon® always be with doctors for the treatment of various liver disease.

- The original silymarin for treatment of liver disease by numerous clinical trials since 1960's.1,2
- Proven efficacy in improvement of liver function:2-11
  - NAFLD, NASH, ALD, cirrhosis
- Multi-therapeutic targets in all-stage of liver disease by various MoA:2-4
  - Improvement of insulin resistance
  - Anti-oxidative stress, Anti-inflammation, Anti-fibrosis
- Good tolerance and safety with lower side effects:5-9

Manufacturer / Distributor
BUKWANG PHARM. CO., LTD.
Seoul, Korea / www.bukwang.co.kr

Technical Partnership
MADIAUS GmbH
Federal Republic of Germany
Go to Europe in 2019

WINUF
Enhanced ω-3 TPN

Lead the direction of Total Parenteral Nutrition

- The highest amount of Fish oil
- ω-6:ω-3=2.1:1 The ideal omega fatty acid composition ratio
- High content of Amino acids
- Provides balanced electrolytes (Zinc, α-tocopherol and other trace elements)
- Completed the first 3-phase clinical case for TPN in Korea
- Acquired the first domestic patent for the globule size stability of lipid emulsion
Effective PI-free treatment means prescribing with confidence despite unknowns1–8,a–c

EPCLUSA® has relatively few clinically relevant DDIs and no limitations around liver disease severity3,c–f

Robust regimen with some forgiveness to non-adherenceg,i

Proven cure rates, even when studied with minimal monitoringg,h

PAN

PAN

Pan-genotypic3,b,g and pan-fibrotic3,b,g

Acknowledged test and treat option3,b,f

Well-characterized on-treatment monitoring in patients with compensated cirrhosis2

Relatively few clinically relevant DDIs3

Pl-free

Suitable despite uncertainties in liver disease severity2,d,k

You can trust EPCLUSA® to deliver consistent outcomes in a variety of settings5,k

sofosbuvir/velpatasvir
400 mg/100 mg tablets

A safe journey for lifelong HBV treatment
The first developed nucleotide analogue in Korea.

**Antiviral effect of Besivo**
- Besivo has antiviral efficacy comparable to that of TDF after 48 weeks of treatment, with durable effects for 192 weeks.

**Safety data of Besivo**
- Besivo has a better safety profile than TDF, in terms of bone and renal outcomes.

**Tolerance of Besivo**
- Besivo had no drug-resistance mutation for 192 weeks.

**Histological effect of Besivo**
- Besivo showed a significantly higher proportion of patients with improved histological scores than TDF.

* TDF: Tenofovir disoproxil fumarate, ** Knodell necroinflammation score

**REFERENCE**

**Besivo® Tab. (Besifovir dipivoxil maleate 183mg (Besifovir dipivoxil 150mg))**

[Indication and Usage] Treatment of chronic hepatitis B in adults

[DOSAGE AND ADMINISTRATION] One tablet containing 150 mg besifovir dipivoxil once daily orally with or without food in adults. When taking this medicine, take 660mg of L-Carnitine together to prevent a decrease in serum L-Carnitine level. (Patients with nephropathy) Patients with mild renal impairment: administration of the drug is not recommended because there is no treatment experience. Patients with moderate, severe renal impairment: It is recommended to administer one tablet once every two days for moderate symptoms and one tablet once every four days for severe symptoms are recommended. Patients with end-stage renal disease: administration of this drug is not recommended because there is no treatment experience.

[WARNINGS AND PRECAUTIONS] 1) Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs in combination with other antiretrovirals. Treatment should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations). 2) Discontinuation of anti-HBV therapy may be associated with severe acute exacerbations of hepatitis. Patients infected with HBV who discontinue Besivo should be closely monitored with both clinical and laboratory follow-up for at least several months after stopping treatment. If appropriate, resumption of anti-hepatitis B therapy may be warranted. 3) HIV-1 antibody testing should be offered to all HBV-infected patients before initiating therapy with Besivo. Limited clinical experience suggests there is a potential for the development of HIV if Besivo is used to treat chronic hepatitis B virus (HBV) infection in patients with HIV infection that is not being treated. Therapy with Besivo is not recommended for HIV/HBV co-infected patients. 4) Since this drug contains lactose, it should not be administered to patients with genetic problems such as galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption.
Liver Fibrosis Single Biomarker

M2BPGi forms in blood when hepatic fibrosis occurs

Collect blood for M2BPGi test

Measure with full automated system

The only single biomarker that is approved reimbursement (Code: D1980)
Pick up only 10μL of serum
Test time 17min
Included in the KASL clinical practical guidelines for managing NAFLD and CHB

Subject & Utility of M2BPGi Test

**Diabetes:** There is a high possibility of advanced hepatic fibrosis with an abnormal M2BPGi level (>1.0).1

**NAFLD patients:** Serum M2BPGi could serve as a reliable biomarker for diagnosing advanced fibrosis and cirrhosis.2

**Liver fibrosis risk population:** Serum M2BPGi has proven to be a dependable, non-invasive surrogate marker for predicting advanced fibrosis.3

**CHB patients receiving long-term antiviral treatment:** The serum M2BPGi level functions as an independent predictor of HCC and complements the stratification of HCC risks.4

**CHB with oral antiviral therapy:** A baseline M2BPGi level above 1.73 consistently demonstrated predictive value for higher HCC risk.4

**TACE treatment for HCC:** The combination of M2BPGi and up-to-seven criteria could serve as a surrogate marker for predicting CP grade deterioration.5

**CHB:** The M2BPGi level can predict HCC development independently.

References

Together for a better healthcare journey

www.sysmex.co.kr
Our heartfelt wish for curing HBV, we present Vemlia.

Comparable antiviral efficacy vs. TDF¹

Improved safety profile in renal and bone parameters²

Increased affordability with lower price, 2,474/tablet³

Improved patients' compliance with daily pill bottle⁴

3. The data above are clinical data conducted with Tenofovir alafenamide hemifumarate.
6. *99$ lower price than Original drug (June 2023)
Maviret® INDICATIONS

MAVIRET treatment duration based on the patient population in HCV mono-infected and HCV/HIV-1 co-infected patients

MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C) or those with any genotype 1, 2, 3, 4, 5, or 6 infection have been taken - advise the patient to take the dose as soon as possible and then to take the next dose at the usual time.

Table 1. Recommended Duration for Treatment-Naïve Patients

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Recommended Treatment Duration</th>
<th>No Cirrhosis</th>
<th>Compensated cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5, or 6</td>
<td>8 weeks</td>
<td>8 weeks</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Recommended Duration for Treatment-Experienced Patients

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>Patients Previously Treated With a Regimen Containing</th>
<th>No Cirrhosis</th>
<th>Compensated cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5, or 6</td>
<td>(Peg)interferon, ribavirin and/or sofosbuvir</td>
<td>8 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>1</td>
<td>An NS3/4A protease inhibitor without prior treatment with an NS5A inhibitor</td>
<td>12 weeks</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>An NS3/4A protease inhibitor with or without ribavirin and/or sofosbuvir</td>
<td>16 weeks</td>
<td></td>
</tr>
</tbody>
</table>

Hepatic Impairment: No dose adjustment of MAVIRET is required in patients with compensated cirrhosis or without cirrhosis and with or without renal impairment including patients receiving dialysis.

ES) Missed Dose: If a dose is missed and it is:

• More than 18 hours from the usual time that MAVIRET should have been taken - advise the patient to take the missed dose and to take the next dose at the usual time.

Disease: Post-marketing cases of hepatic decompensation/failure, including those with fatal outcomes, have been reported in patients treated with HCV NS3/4A protease inhibitor-containing regimens, including MAVIRET. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. The majority of patients with severe outcomes had evidence of advanced liver disease with moderate or severe hepatic impairment (Child-Pugh B or C) prior to starting therapy with MAVIRET, including some patients reported as having compensated cirrhosis with mild liver impairment (Child Pugh A) at baseline but with a prior decompensation event (i.e., prior history of ascites, variceal bleeding, encephalopathy). Rare cases of hepatic decompensation/failure were reported in patients without cirrhosis or with compensated cirrhosis (Child-Pugh A). Many of these patients had evidence of portal hypertension. Events also occurred in patients taking a concomitant medication not recommended for coadministration, or in patients with comorbid factors such as severe liver or renal or surgical complications. Cases typically occurred within the first 6 weeks of treatment (median of 27 days) in patients with compensated cirrhosis (Child-Pugh A) or evidence of advanced liver disease such as portal hypertension, perform hepatic laboratory testing as clinically indicated and monitor for signs and symptoms of hepatic decompensation such as guarding, ascites, hepatic encephalopathy, and variceal hemorrhage. Discontinue MAVIRET in patients who develop evidence of hepatic decompensation/failure. MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C) in those with any history of prior hepatic decompensation. Due to this risk, MAVIRET is recommended in patients with mild or moderate impairment not taking medications that are known to cause hepatic decompensation. Medications known to cause hepatic decompensation include but are not limited to: (i) azathioprine, anthracyclines, methotrexate, allopurinol, anthracyclines, valproic acid, amphotericin B, and retinoids; (ii) steroids; (iii) QT-prolonging agents; (iv) diuretics and thiazide diuretics; and (v) other medications known to cause hepatic decompensation. Any new medication or dose adjustment of a medication modified when necessary. The physician in charge of the diabetic care of the patient should be informed.

Drug Interactions: At a dose adjustment of MAVIRET is required in patients with mild hepatic impairment (Child-Pugh A). MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation. Due to this risk, MAVIRET is recommended in patients with mild, moderate, or severe hepatic impairment. Non-clinical studies indicate that MAVIRET is generally well tolerated in healthy volunteers and patients with mild, moderate or severe hepatic impairment. A clinical trial conducted in patients with genotype 1 infection who were HCV mono-infected or without prior treatment with an NS5A inhibitor or with genotype 1 infection who were HCV/HIV-1 co-infected included patients with mild, moderate, or severe hepatic impairment, including some patients reported as having compensated cirrhosis with mild liver impairment (Child Pugh A) at baseline but with a prior decompensation event (i.e., prior history of ascites, variceal bleeding, encephalopathy). Rare cases of hepatic decompensation/failure were reported in patients without cirrhosis or with compensated cirrhosis (Child-Pugh A). Many of these patients had evidence of portal hypertension. Events also occurred in patients taking a concomitant medication not recommended for coadministration, or in patients with comorbid factors such as severe liver or renal or surgical complications. Cases typically occurred within the first 6 weeks of treatment (median of 27 days) in patients with compensated cirrhosis (Child-Pugh A) or evidence of advanced liver disease such as portal hypertension, perform hepatic laboratory testing as clinically indicated and monitor for signs and symptoms of hepatic decompensation such as guarding, ascites, hepatic encephalopathy, and variceal hemorrhage. Discontinue MAVIRET in patients who develop evidence of hepatic decompensation/failure. MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C) in those with any history of prior hepatic decompensation. Due to this risk, MAVIRET is recommended in patients with mild or moderate impairment not taking medications that are known to cause hepatic decompensation. Medications known to cause hepatic decompensation include but are not limited to: (i) azathioprine, anthracyclines, methotrexate, allopurinol, anthracyclines, valproic acid, amphotericin B, and retinoids; (ii) steroids; (iii) QT-prolonging agents; (iv) diuretics and thiazide diuretics; and (v) other medications known to cause hepatic decompensation. Any new medication or dose adjustment of a medication modified when necessary. The physician in charge of the diabetic care of the patient should be informed.
Gilead Liver Commitment
Exploring for Complete Understanding of Liver Disease
VEMLIDY-for the flow of life with chronic hepatitis B


QR 코드를 스캔하여
Vemlidy® 키를 확인하십시오.
Recognize & Kill the cancer cells

Immuncell-LC
Anticancer cellular Immunotherapeutics

ANTI-CANCER  SAFETY  QUALITY of LIFE

Received approval for cancer immunotherapy ‘Immuncell-LC’ from MFDS in 2007


Efficacy-Effect: Adjunctive therapy for patients whose tumor has been removed after curative resection for Hepatocellular Carcinoma (Operation, Radio Frequency Ablation, Percutaneous Ethanol Injection Therapy)

Dosage and Administration: Mix the settled cells and suspension fluid three or four times prior to administration. The interval and times of administration are as follows: 4 times, once a week, 4 times, once every two weeks, 4 times, once four weeks, 4 times, once every eight weeks 16 times in total.
The new wave of GERD Treatment, P-CAB

**FEXUSLUE**
Fexuprazan hydrochloride

- Excellent nocturnal symptom control: Longest half-life
- Significantly improved chronic cough of EE
- Take once a day regardless of meal
- Rapid and superior heartburn symptom relief
- Full and fast onset of effect with the first dose
- Less affected by CYP2C19: Low potential of DDI individual variations
FibroScan®
by echosens

The non-invasive gold standard solution for comprehensive management of liver health

CAP 신의료기술 고시
보건복지부 고시 제2021-163호, 2021.6.7

New! Fibroscan 630 Expert
Spleen Stiffness Measurement (SSM by VCTE) Surrogate marker of PH

Scores (Agile 3+ & 4) by Echosens
LSM과 혈액 바이오 마커(AST, ALT, Plt) 결합 및 계산하여 NAFLD 환자의 F3/F4를 식별합니다

Surrogate marker of liver fibrosis
- Measurement of liver stiffness (expressed in kPa)
- Relevant in all Chronic Liver Diseases (CLD)

Surrogate marker of liver steatosis
- Measurement of ultrasound attenuation (expressed in dB/m)
- Relevant in Fatty Liver Diseases: AFLD, NAFLD, NASH

Surrogate marker of portal hypertension (PH)
- Shear wave frequency of 100Hz
- Relevant in the diagnosis of large esophageal varices
- Relevant for the risk stratification of cirrhotic patients
Confidence for NAFLD treatment
Evidenced by numerous clinical results

GODEX® cap.

- Restoration of Hepatic Mitochondrial Dysfunction by Carnitine Complex
- Rapid Normalization of ALT Level
- Improving effect for NAFLD as Evidenced by CT scans

Product Information
- Description: Reddish brown colored hard gelatin capsule containing yellowish brown colored powder.
- Composition: Each capsule contains Carnitine Orotate 150mg (73.8mg as orotic acid, 76.2mg as carnitine), Liver Extract Antioxidant fraction 12.5mg, Adenosine HC2 25mg, Pyridoxine HC2 25mg, Riboflavin 0.5mg, Cyanocobalamin 0.125mg, Biphenyl dimethyl dicarboxylate 25mg.
- Indication: General therapeutics for the following hepatic disease: - Acute, Subacute and Chronic Hepatitis, Hepatic cirrhosis, Fatty liver, Drug or chemical induced hepatitis. 2) Acute, chronic hepatitis involving high transaminase value.
- Dosage & Administration: Usually, each time 2 capsules, 2-3 times a day as adult dosage. Dosage unit can be changeable depending on symptom or age of patient.
- Special caution: 1) Severe state of chronic hepatitis. 2) Severe state of hepatic cirrhosis.
- General caution: 1) Rarely skin rash can be represented, in this case, discontinuation of medical use is necessary. 2) In severe case, sometimes intermittent jaundice can be occur; in this case, discontinuation of medical use is necessary. 3) Rarely nausea, gastric discomfort can be represented.
- Packaging: 100, 300 caps. (bottle)/ 100 caps. (PTP)
- Storage: Tight closed container, room temperature (15-30°C) in dry place. Expiry - 60 months from Manufacturing date.

Diagnostic Codes
- B15-19 Viral hepatitis
- K70.0 Alcoholic fatty liver
- K71.0 Toxic liver disease
- K73.0 Chronic persistent hepatitis, NEC
- K74.0 Hepatic fibrosis
- K75.8 Other specified inflammatory liver disease
- K77.0 Nonalcoholic steatohepatitis
- Liver disorders in disease classified elsewhere
NEXT PIECE FOR BEST PEACE

Experience a better tomorrow with VEMLINO

VEMLINO, Effective for early stage and impaired renal function or decreased bone mineral density of hepatitis B patients.
Obtained ‘Exclusive Marketing Rights’!
First Generic of Sorafenib

Soranib was officially approved by MFDS on October 29th, 2020.

Treatment of hepatocellular carcinoma, thyroid carcinoma and renal cell carcinoma

Soranib Tab. 200mg on Market!
(Soranib tosylate(Micronized))
(since December 1st, 2020.)

1. Obtained ‘exclusive marketing rights’
   by demonstrating bioequivalence to the original product
2. Accumulated more than 10 years of experience in prescribing Sorafenib
3. The First-generic to ease the burden of medication cost
4. Improved patient convenience by redesigning the package

I had the will to start a business from scratch. But I still need help to lose weight and keep it off.

SARAH, Age 43, BMI 37 (Patient portrayal)

For people with obesity, losing weight and keeping it off is more than a matter of willpower. Changes in appetite-regulating hormones after weight loss drive weight regain, undermining their efforts.

Saxenda® is 97% similar to natural GLP-1, a hormone that works in the brain to decrease appetite and thereby reduce food intake, leading to significant and sustained weight loss.2,3

Obesity is a chronic disease, and most patients want your help. Ask your patients about their weight loss attempts, and tell them how adding Saxenda® to diet and exercise can help them lose weight and keep it off.2

Your patients with obesity have the will. You can offer them the way.

Saxenda® injection

Remarkable Response

The ORR was more than three times higher with lenvatinib versus control group. Based on the masked IIR according to mRECIST, about 41% of patients showed ≥ 30% decrease in tumor size.

40.6%* Response Rate
(Masked IIR according to mRECIST)

* ORR is one of the secondary endpoints and this is the result of the post hoc exploratory tumour assessments using mRECIST by masked central independent imaging review. For more information, please refer to the full article (Kudo M, et al. 2018).

<table>
<thead>
<tr>
<th></th>
<th>Lenvatinib (n=478)</th>
<th>Sorafenib (n=476)</th>
<th>Effect size (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator review according to mRECIST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective response (% 95% CI)</td>
<td>115 (24.1%, 20.2-27.9)</td>
<td>44 (9.2%, 6.6-11.8)</td>
<td>OR 3.13 (2.13-4.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Masked independent imaging review according to mRECIST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective response (% 95% CI)</td>
<td>194 (40.6%, 36.2-45.0)</td>
<td>59 (12.4%, 9.4-15.4)</td>
<td>OR 5.01 (3.59-7.01)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Masked independent imaging review according to RECIST 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective response (% 95% CI)</td>
<td>90 (18.8%, 15.3-22.3)</td>
<td>31 (6.5%, 4.3-8.7)</td>
<td>OR 3.34 (2.17-5.14)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

mRECIST: modified Response Evaluation Criteria in Solid Tumors, IIR: Independent Imaging review, CI: Confidence Interval, OR: Odds Ratio

References:
Kudo M et al. Lancet, 2018 Mar 24;391(10094):1196-1207. Lenvima® (lenvatinib mesylate) tablets 20mg: Lenvima® (lenvatinib mesylate) tablets 10mg: Lenvima® (lenvatinib mesylate) tablets 5mg: Lenvima® (lenvatinib mesylate) capsules 20mg: Lenvima® (lenvatinib mesylate) capsules 10mg: Lenvima® (lenvatinib mesylate) capsules 5mg: Lenvima® (lenvatinib mesylate) powder for oral suspension 5mg/mL: Lenvima® (lenvatinib mesylate) powder for oral suspension 2.5mg/mL: Lenvima® (lenvatinib mesylate) powder for oral suspension 1.25mg/mL: Lenvima® (lenvatinib mesylate) powder for oral suspension 0.625mg/mL: Lenvima® (lenvatinib mesylate) powder for oral suspension 0.3125mg/mL: EMA: European Medicines Agency, FDA: US Food and Drug Administration, NICE: National Institute for Health and Care Excellence. Eisai shares information on the website in accordance with applicable laws and regulations.
Ramnos® not only strengthens intestinal health and immunity, but also improves atopic symptoms.
Damaged Livers Can Be Recovered

The Only Korean Medicine Proven to Reduce the Level of MDA, a Biomarker of Oxidative Stress, Through Phase IV Clinical Trials Significantly Reduced the Level of MDA in Alcoholic Hepatitis, Nonalcoholic Steatohepatitis and Viral Hepatitis Patients

Safe Medicine Proven to Improve Quality of Life for Patients
Patients’ Improved Quality of Life Verified Through Chronic Liver Disease Questionnaire (CLDQ)

Antioxidative Effect Reduces Fat in the Liver Proven to Reduce MDA Level

Proven Efficacy
Quickly Reduces and Helps You Maintain Optimal Level of Alanine Transaminase (ALT)
Contains Garlic Oil Which is Known to Have Strong Antioxidative and Anti-Inflammatory Effects

PENNEL

Ingredients: Chronics hepatitis with continuously elevated ALT level
Directions: Take 1 or 2 capsules each time, 3 times a day, after meals

Diagnostic Code
B15–19 Viral hepatitis K70.0 Alcoholic fatty liver K71.0 Toxic liver disease K73.0 Chronic persistent hepatitis, NEC K74.0 Hepatic fibrosis K75.8 Other specified inflammatory liver disease, Nonalcoholic steatohepatitis K77.0 Liver disorders in disease classified elsewhere
SK Albumin Inj. 5%/20%

Human serum albumin

» Maintenance of Intravascular pressure
» Acid-base balance
» Drug transport
» Transport of ions, fatty acids, bilirubin and hormones

SK plasma

ECO Lata, 310 Pannyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea
Tel: +82-32-800-0028 www.skplasma.com
Your precision strike. 
Arming you to target HCC tumors directly and hit them hard with high-dose radiation therapy.

Proven. 
Personalized. 
Precise.
**Oral Suspension**

**Megace® F**
(megestrol acetate, USP)

Reformulation of MG OS by utilizing NanoCrystal® Technology. 50 times smaller particles increased surface area

**rapid dissolution & increased absorption.**

**Improved bioavailability**

I. **Originality**
- licensed the Megace® name from Bristol-Myers Squibb Company
- High Quality

II. **Improved Bioavailability!**

*** Food effect differences between a nanocrystal dispersion of megestrol acetate 625 mg/5 mL and a micronized formulation of megestrol acetate oral suspension (MGOS) 800 mg/20 mL. ***

![Graph 1: Fed condition](image1)

![Graph 2: Fasting condition](image2)

In-house data (Boryung Pharm)

III. **Improved Efficacy!**
- Weight gain occurred more rapidly at each time point
- Patients in the nanocrystal dispersion arm gained an average of 10% of the baseline weight over 12 weeks
  (Vs 6% weight gain in MG OS arm)

IV. **Improved Convenience!**

<table>
<thead>
<tr>
<th></th>
<th>Megace F-OS</th>
<th>Megace-OS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended dose</strong></td>
<td>1 teaspoon</td>
<td>4 teaspoon</td>
</tr>
<tr>
<td>625 mg/5mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 mg/20mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Viscosity</strong></td>
<td>10 cP*</td>
<td>163 cP*</td>
</tr>
</tbody>
</table>

* cP = centipoise, a measure of viscosity, with higher numbers indicating greater viscosity.

International Journal of Nanomedicine 2009:4 185-192