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.
Reviews

1  Recent advances in the management of hepatocellular carcinoma
Kamya Sankar, Jun Gong, Arsen Osipov, Steven A. Miles, Kambiz Kosari, Nicholas N. Nissen, Andrew E. Hendifar, Ekaterina K. Koltsova, and Ju Dong Yang

16  Taiwan Association for the Study of the Liver-Taiwan Society of Cardiology Taiwan position statement for the management of metabolic dysfunction-associated fatty liver disease and cardiovascular diseases

Original Articles

37  Comparison of four histological scoring systems for autoimmune hepatitis to improve diagnostic sensitivity
Soomin Ahn, Sook-Hyang Jeong, Eun Ju Cho, Kyoungbun Lee, Gilhyang Kim, and Haeryoung Kim

49  Cardiovascular risk in chronic hepatitis B patients treated with tenofovir disoproxil fumarate or tenofovir alafenamide
Hyeyeon Hong, Won-Mook Choi, Danbi Lee, Ju Hyun Shim, Kang Mo Kim, Young-Suk Lim, Han Chu Lee, and Jonggi Choi

64  Artificial intelligence predicts direct-acting antivirals failure among hepatitis C virus patients: A nationwide hepatitis C virus registry program

80  Protein-centric omics analysis reveals circulating complements linked to non-viral liver diseases as potential therapeutic targets
Yingzhou Shi, Hang Dong, Shiwei Sun, Xiaojin Wu, Jiansong Fang, Jianbo Zhao, Junming Han, Zongyue Li, Huixiao Wu, Luna Liu, Wanhong Wu, Yang Tian, Guandou Yuan, Xiude Fan, and Chao Xu
Hepatitis B core-related antigen dynamics and risk of subsequent clinical relapses after nucleos(t)ide analog cessation
Ying-Nan Tsai, Jia-Ling Wu, Cheng-Hao Tseng, Tzu-Haw Chen, Yi-Ling Wu, Chieh-Chang Chen, Yu-Jen Fang, Tzeng-Huey Yang, Mindie H. Nguyen, Jaw-Town Lin, and Yao-Chun Hsu

Letter to the Editor
109 Letter regarding “Treated chronic hepatitis B is a good prognostic factor of diffuse large B-cell lymphoma”
Chi Hsiao and Yung-Po Liaw

111 Letter 1 regarding “Assessing the performance of ChatGPT in answering questions regarding cirrhosis and hepatocellular carcinoma”
Hinpetch Daungsupawong and Viroj Wiwanitkit

113 Letter 2 regarding “Assessing the performance of ChatGPT in answering questions regarding cirrhosis and hepatocellular carcinoma”
Yiwen Zhang, Liwei Wu, Zepeng Mu, Linlin Ren, Ying Chen, Hanyun Liu, Lili Xu, Yangang Wang, Yaxing Wang, Susan Cheng, Yih Chung Tham, Bin Sheng, Tien Yin Wong, and Hongwei Ji

118 Letter regarding “Waiting for the changes after the adoption of steatotic liver disease”
Kuo Chao Yew, Sunny H. Wong, Vincent Wai-Sun Wong, and Hazel H. Oon

121 Changing from NAFLD to MASLD: Similar cumulative incidence of reflux esophagitis between NAFLD and MASLD
Shuhei Fukunaga, Michita Mukasa, Dan Nakano, Tsubasa Tsutsumi, and Takumi Kawaguchi

Correspondence
124 Correspondence on Letter regarding “Assessing the performance of ChatGPT in answering questions regarding cirrhosis and hepatocellular carcinoma”
Yee Hui Yeo and Ju Dong Yang

126 Correspondence on Letter regarding “Waiting for the changes after the adoption of steatotic liver disease”
Eileen L. Yoon and Dae Won Jun

 Snapshot
129 Recent updates on pharmacologic therapy in nonalcoholic fatty liver disease
Young Chang, Soung Won Jeong, and Jae Young Jang
Erratum to 'Correspondence on Letter regarding “Evidence-based hyponatremia management in liver disease”’ [Clin Mol Hepatol 2023;29:1048-1049]

Seon Ha Baek, Ji Young Ryu, and Sejoong Kim
Recent advances in the management of hepatocellular carcinoma

Kamya Sankar¹, Jun Gong¹, Arsen Osipov¹, Steven A. Miles¹, Kambiz Kosari¹², Nicholas N. Nissen¹², Andrew E. Hendifar¹, Ekaterina K. Koltsova³, and Ju Dong Yang¹²⁴

¹Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center; ²Comprehensive Transplant Center, Cedars-Sinai Medical Center; ³Smidt Heart Institute, Cedars-Sinai Medical Center; ⁴Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Liver cancer remains a challenge of global health, being the 4th leading cause of cancer death worldwide. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, and is usually precipitated by chronic viral infections (hepatitis B and C), non-alcoholic steatohepatitis, heavy alcohol use, and other factors which may lead to chronic inflammation and cirrhosis of the liver. There have been significant advances in the systemic treatment options for HCC over the past decades, with several approvals of both immune checkpoint inhibitors and tyrosine kinase inhibitors in patients with preserved liver function. These advances have led to improvement in survival outcomes, with expected survival of greater than 18 months, in those with sensitive tumors, adequate liver function, and those functionally fit to receive sequential therapies. Several ongoing and promising trials are now evaluating combinational strategies with novel systemic agents and combinations of systemic therapy with locoregional therapy. In view of these trials, further advances in the treatment of HCC are foreseen in the near future. (Clin Mol Hepatol 2024;30:1-15)

Keywords: Hepatocellular carcinoma; Liver cancer; Immunotherapy; Tyrosine protein kinase inhibitors

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, encompassing 80–90% of primary liver cancers. Despite significant advances in therapeutics, HCC has high mortality rates in the United States and globally.¹² HCC is the 6th most common cancer and 4th leading cause of cancer-related death globally, and thus a significant affliction to public health.³ Chronic hepatic inflammation and liver cirrhosis from any cause is the strongest risk factor for HCC. Chronic inflammation may arise from heavy alcohol use, nonalcoholic steatohepatitis (NASH), viral infections (hepatitis B [HBV] and hepatitis C [HCV]), chronic toxin exposure (e.g., aflatoxin). Lifestyle factors like chronic alcohol consumption, dietary

Corresponding author: Kamya Sankar
Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, 8900 Beverly Blvd, Los Angeles, CA 90048, USA
Tel: +1-310-423-1840, Fax: +1-310-659-3928, E-mail: kamya.sankar@cshs.org
https://orcid.org/0000-0001-8636-8479

Ju Dong Yang
Cedars-Sinai Medical Center, 8900 Beverly Blvd, Los Angeles, CA 90048, USA
Tel: +1-310-423-1971, Fax: +1-310-423-2356, E-mail: judong.yang@cshs.org
https://orcid.org/0000-0001-7834-9825

Editor: Naoshi Nishida, Kindai University, Japan
Received: Mar. 26, 2023 / Revised: Jul. 18, 2023 / Accepted: Jul. 18, 2023

Copyright © 2024 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.
habits, and sedentary lifestyle, have led to a continued rise in the incidence of HCC despite advances in anti-viral therapies in dampening HBV and HCV related cirrhosis.3,4 Diagnosis of HCC remains largely based on radiologic findings.5 This is in the setting of increased need for better molecular characterization of the disease which requires either tissue for analysis or liquid biopsy, or sometimes both. Immune checkpoint inhibitors (ICI) blocking programmed death ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1) have transformed the treatment landscape for HCC and now form the backbone of most systemic therapies in clinical practice and in trials. Several systemic options have been approved for first-line therapies (atezolizumab and bevacizumab [atezo-bev], durvalumab and tremelimumab [durva-treme], sorafenib, lenvatinib). In addition, several second-line agents such as regorafenib, cabozantinib, ramucirumab, nivolumab with ipilimumab, and pembrolizumab are now approved and available. Many ongoing trials are investigating novel strategies involving tyrosine kinase inhibitors in combination with ICI, locoregional treatment in combination with ICI, and ICI combination strategies in the neoadjuvant setting. Herein, we review the current approaches to the management of advanced HCC. We discuss the evolution of systemic therapy for HCC, strategies for treatment selection and sequencing, clinical challenges in the treatment of HCC, and future directions for novel therapeutic strategies for HCC.

APPROACH TO MANAGEMENT

The relatively recent availability of multiple systemic options in the first and subsequent line settings have significantly changed the treatment landscape for HCC. Most patients with HCC have underlying cirrhosis, which significantly impacts their health, performance status, and ability to tolerate surgical, locoregional and systemic treatments. As a result, treatment must be individualized.5 Currently, there is a widespread global consensus among clinicians to base treatment of HCC on the tumor stage based on the Barcelona Clinic Liver Cancer (BCLC) staging system.7,8 Patients who have early-stage HCC (BCLC 0 or BCLC A) are candidates for treatment with surgical resection, ablation or liver transplant.9 Patients with intermediate-stage HCC (BCLC B) are treated with locoregional therapies such as trans-arterial chemoembolization (TACE), trans-arterial radioembolization with yttrium-90 (Y90), and/or systemic therapy.5 Several randomized studies are ongoing evaluating the combination of systemic treatment with locoregional treatment for intermediate-stage HCC (NCT04246177, NCT03778957, NCT04340193, NCT04268888). Patients with advanced-stage HCC (BCLC C) are treated with systemic therapies upfront. Select patients with BCLC stage B and rarely C can become candidates for liver transplant with adequate downstaging.

Prognosis of patients with HCC correlates well with their BCLC stage. Median survival ranges from greater than 10 years in patients who receive liver transplant for early stage, to more than 6 years for patients who undergo resection or ablation, approximately 26–30 months for patients with intermediate-stage HCC, and approximately 19 months in patients with advanced stage with compensated liver function (Child Pugh A cirrhosis).5 These outcomes are significantly improved in the era of immunotherapy as compared to historic data in patients with HCC.10

SYSTEMIC THERAPIES

In treatment-naïve patients with unresectable HCC, sorafenib was first shown to prolong survival compared to placebo.11 Lenvatinib has been shown to be non-inferior to sorafenib.12 Both atezo-bev and durva-treme improved survival compared to sorafenib.13,14 Finally, durvalumab monotherapy has been shown to be non-inferior to sorafenib.14 A summary of the currently approved systemic therapies for the management of advanced HCC is shown in Figure 1.

Abbreviations:
HCC, hepatocellular carcinoma; NASH, nonalcoholic steatohepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus; ICI, immune checkpoint inhibitors; PD-L1, programmed death ligand 1; PD-1, programmed cell death protein 1; BCLC, Barcelona Clinic Liver Cancer; TACE, trans-arterial chemoembolization; Y90, yttrium-90; TKI, tyrosine kinase inhibitor; OS, overall survival; PFS, progression-free survival; CTLA-4, cytotoxic T-lymphocyte antigen-4; RT, radiotherapy; TARE, transarterial radioembolization; LEN, lenvatinib
Currently approved tyrosine-kinase inhibitors and anti-angiogenic agents for treatment of HCC

Prior to 2007, there were no standard systemic therapies for HCC. Cytotoxic chemotherapy was used with limited benefit with high rates of toxicity often stemming from underlying pre-existing liver dysfunction in treated patients. The SHARP trial in 2007 was the first clinical trial to show a survival benefit in HCC where sorafenib, a tyrosine kinase inhibitor (TKI), improved median overall survival (OS) from 7.9 months to 10.7 months when compared to placebo (hazard ratio [HR] 0.69, 95% confidence interval [CI] 0.55–0.87; \( P < 0.001 \)).

Lenvatinib, a multi-kinase TKI (targeting VEGF receptor 1–3, FGF receptor 1–4, PDGF receptor alpha, RET, and KIT), was then studied in comparison to sorafenib in the REFLECT trial. Median OS in the lenvatinib group was non-inferior to sorafenib (median OS 13.6 vs. 12.3 months, HR 0.92, 95% CI 0.79–1.06). Lenvatinib had a higher objective response rate (18.8% vs. 6.5%), time-to-progression (7.4 vs. 3.7 months), and median progression-free survival (PFS) (7.3 vs. 3.6 months). There was a higher incidence of treatment-related adverse events in the lenvatinib arm (43% vs. 30%). Lenvatinib was approved by the US Food and Drug Administration (FDA) in 2018 as the first-line treatment for patients with advanced/unresectable HCC.

In the subsequent-line settings, both cabozantinib and regorafenib are approved. Regorafenib was studied in the RESORCE study which randomized HCC patients who previously progressed on sorafenib with Child Pugh A cirrhosis 2:1 to receive regorafenib or placebo. Regorafenib improved OS (median OS 10.6 vs. 7.8 months, HR 0.63, \( P < 0.0001 \)). The objective response rate for regorafenib was 7% with median duration of response of 3.5 months. Grade 3 or 4 adverse events were reported in 46% of patients. Regorafenib was approved in 2017 for treatment of HCC after progression on sorafenib.

Cabozantinib, a multi-kinase TKI that targets VEGF receptors 1–3, MET and AXL, was studied in the CELESTIAL trial where patients with HCC who had disease progression after 1–2 systemic treatments were randomized 2:1 to receive cabozantinib or placebo. Cabozantinib treated patients showed improved median OS (10.2 vs. 8 months, HR 0.76, 95% CI 0.63–0.92, \( P = 0.005 \)) and median PFS (5.2 vs. 1.9 months, HR 0.44, 95% CI 0.36–0.52, \( P < 0.001 \)). The objective response rate was 4%. Grade 3 or 4 adverse events occurred in 68% of patients in the cabozantinib group. In 2019, cabozantinib was approved for patients with HCC who had previously been treated with sorafenib.

Ramucirumab, a monoclonal antibody which targets VEGF receptor 2, was studied in a placebo-controlled randomized trial in patients with BCLC stage B or C HCC who had shown...
progression on sorafenib and had alpha-fetoprotein concentrations of 400 ng/mL or greater. This study met its primary endpoint where ramucirumab improved median OS (8.5 vs. 7.3 months, HR 0.71, 95% CI 0.53–0.95, P=0.019) and median PFS (2.8 vs. 1.6 months, HR 0.45, 95% CI 0.34–0.60, P<0.0001). The objective response rate did not significantly differ between the two groups (5% vs. 1%). The currently approved TKI and anti-angiogenic agents are summarized in Table 1.

Currently approved checkpoint inhibitors in treatment of HCC

The immunotherapy era in the management of HCC began after a pilot study published in 2013 showed the safety and anti-tumor activity of tremelimumab, an inhibitor of cytotoxic T-lymphocyte antigen-4 (CTLA-4), in patients who developed HCC with HCV cirrhosis. In the ensuing decade, the availability of immunotherapy as a treatment option for HCC has had a tremendous impact in the field as demonstrated by its adoption and inclusion of immunotherapy in the majority of treatment algorithms for HCC in clinical practice. Furthermore, most if not all systemic treatments being evaluated in randomized phase III studies in advanced HCC involve an ICI backbone. However, despite major advances and a shift in the treatment paradigm, only a fraction of patients respond to ICI, particularly as monotherapy, thus highlighting the importance of research in biomarker driven strategies and combination approaches.

Single agents

Nivolumab and pembrolizumab showed activity in phase II trials that evaluated their role as second-line agents when used after progression on sorafenib. These studies showed a response rate of 15–20% (complete response rate of 1–5%) which were durable. In the CheckMate 040 trial, the 2 year survival rate among responders to nivolumab was over 80%. Based on these data, both nivolumab and pembrolizumab obtained accelerated approval by regulatory agencies as second-line treatment after progression on or unacceptable toxicity to sorafenib.

CheckMate 459 was a randomized phase III study evaluating nivolumab compared to sorafenib in treatment-naïve patients with advanced HCC and Child Pugh A cirrhosis. At a median follow up of 15.2 months, a trend towards improved OS in the nivolumab arm was reported (median 16.4 vs. 14.7 months, HR 0.85, P=0.075). Further, patients who received nivolumab had improved durable disease control (median 7.5 vs. 5.7 months) and improved toxicity profile with fewer grade 3 or 4 treatment-related adverse events (22% vs. 49%). However, due to not achieving statistical significance for the primary endpoint of OS, the FDA withdrew its approval of nivolumab for treatment of advanced HCC in 2021.

In a relatively recent randomized phase III placebo-controlled study of patients with advanced HCC previously treated with sorafenib (KEYNOTE-240) pembrolizumab resulted in a median OS of 13.9 months vs. 10.6 months in placebo, objective response rate of 18.3% (vs. 4.4%), and grade 3 or higher treatment-related adverse events rate of 52.7% (vs. 46.3%). However, statistical significance for improvement in OS was not reached. Pembrolizumab is a category 2B recommendation in the second-line setting for patients with advanced HCC after progression on TKIs. Another randomized phase III study (KEYNOTE-394) which randomized patients in Asia with advanced HCC with progression on or intolerance to sorafenib to either pembrolizumab or placebo, showed an improvement in OS, PFS, and objective response rate in patients who received pembrolizumab. Overall the results were supportive of the use of pembrolizumab as second-line therapy for advanced HCC.

Combination approaches

The promising activity and favorable safety profile of single-agent ICIs in the management of HCC has spurred the evaluation of various combination strategies, some of which are already being used in clinical practice.

The combination of PD-L1 inhibitor atezolizumab and VEGF inhibitor bevacizumab (atezo-bev) evaluated in the IMbrave150 clinical trial established a new standard of care in 2020 for first-line treatment of patients with unresectable HCC after more than a decade of failing clinical trials. IMbrave150 evaluated sorafenib versus atezo-bev in treatment-naïve patients with unresectable HCC. Compared to sorafenib, Atezo-bev improved both OS and PFS (median OS NE vs. 13.2 months, HR 0.58, P<0.001; median PFS 6.8 vs. 4.5 months, HR 0.59, P<0.001). Atezo-bev also improved objective response rate (27.3% vs. 11.9%, P<0.001) with more durable responses (duration >6 months in 87.6% vs. 59.1% of pa-
Table 1. Landmark trials evaluating TKIs and anti-angiogenic agents for systemic therapy in HCC

<table>
<thead>
<tr>
<th>Trial (reference)</th>
<th>Sample size (n)</th>
<th>Inclusion criteria</th>
<th>Phase and comparator</th>
<th>Primary endpoint(s)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHARP[11]</td>
<td>602</td>
<td>Patients with advanced HCC without prior systemic therapy, with ECOG PS 0-2 and Child-Pugh liver function class A</td>
<td>3</td>
<td>Overall survival</td>
<td>Median OS 10.7 vs. 7.9 months (HR 0.69; ( P &lt; 0.001 ))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sorafenib 400 mg of twice daily or placebo</td>
<td>Time to symptomatic progression</td>
<td>Median TTP 4.1 vs. 4.9 months (HR 1.08; ( P = 0.77 ))</td>
</tr>
<tr>
<td>REFLECT[12]</td>
<td>1,492</td>
<td>Unresectable HCC with measurable target lesions, BCLC stage B or C, Child-Pugh Class A, and ECOG 0-1</td>
<td>3</td>
<td>Overall survival</td>
<td>Median OS 13.6 vs. 12.3 months (HR 0.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lenvatinib 8 mg or 12 mg based on body weight or sorafenib 400 mg twice daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RESORCE[13]</td>
<td>843</td>
<td>Patients with BCLC stage B or C HCC not eligible for local treatments with documented radiologic progression during sorafenib treatment, with Child-Pugh class A liver function.</td>
<td>3</td>
<td>Overall survival, analyzed by intention to treat</td>
<td>Median OS 10.6 vs. 7.8 months (HR 0.63; ( P &lt; 0.001 ))</td>
</tr>
<tr>
<td>CELESTIAL[14]</td>
<td>707</td>
<td>HCC not amenable to curative treatment with Child-Pugh class A liver function with up to 2 lines of prior treatment for HCC (including sorafenib) with ECOG PS 0-1</td>
<td>3</td>
<td>Overall survival</td>
<td>Median OS 10.2 vs. 8.0 months (HR 0.76; ( P = 0.005 ))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cabozantinib 60 mg daily or matching placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REACH-2[15]</td>
<td>292</td>
<td>Patients with BCLC stage B or C HCC treated with prior sorafenib, with AFP ( \geq 400 ) ng/mL</td>
<td>3</td>
<td>Overall survival</td>
<td>Median OS 8.5 vs. 7.3 months (HR 0.7; ( P = 0.0199 ))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ramucirumab 8 mg/kg or placebo every 14 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TKI, tyrosine kinase inhibitor; HCC, hepatocellular carcinoma; ECOG ECOG PS, Eastern Cooperative Oncology Group Performance status; AFP, alpha fetoprotein; BCLC, Barcelona Clinic Liver Cancer; OS, overall survival; HR, hazard ratio.
patients). The most recent analysis shows a median OS of 19.3 months in patients who received aezo-bev and 13 months in patients who received sorafenib (HR 0.66, P<0.001). Furthermore, health-related quality of life was also significantly improved in the aezo-bev arm where the median time to deterioration in patient-reported quality of life was longer with the combination (11.2 vs. 3.6 months; HR 0.63). Atezo-bev was approved by the FDA in 2020 for treatment-naïve patients with unresectable or advanced HCC. A global observational study evaluated 433 patients who received aezo-bev in the first line setting for advanced HCC across Europe, Asia, and the United State. At a median follow up of 10 months, the median OS was 15.7 months, median PFS 6.9 months, and overall response rate 30.8%. While this study confirmed reproducible safety and efficacy of aezo-bev in a real world population with results comparable to that of IMBrave150, the median OS was noted to be shorter than that reported in IMBrave150. It is possible that the patient population, with a higher proportion of patients who demonstrated portal vein thrombus and extrahepatic spread in addition to higher albumin-bilirubin grade may have contributed to this finding. The authors reported that within patients with Child Pugh A criteria, the presence of portal vein thrombosis (PVT) and higher albumin-bilirubin grade was associated with poor survival.

The combination of CTLA-4 and PD-1 blockade was studied in the HIMALAYA trial, leading to another approval in the first-line setting in 2022. In this study, patients with unresectable treatment-naïve HCC were randomized to receive tremelimumab 300 mg (one dose) plus durvalumab 1,500 mg every 3 weeks (STRIDE), durvalumab 1,500 mg every 4 weeks, or sorafenib 400 mg BID. Patients who received STRIDE had a higher median OS (16.4 vs. 16.56 vs. 13.77 months; HR 0.78, p=0.0035). OS with durvalumab alone was noninferior to sorafenib (HR 0.86, non-inferiority margin, 1.08). Grade 3–4 treatment-related adverse events occurred in 50.5% of patients who received STRIDE, 37.1% of patients who received durvalumab and 52.4% of patients who received sorafenib. The combination of durvalumab and tremelimumab is now an approved first-line regimen for patients with advanced HCC. Durvalumab monotherapy can be considered in patients who are not candidates for combination ICI or anti-angiogenic agents.

In CheckMate 040 (phase 1/2 study), patients with advanced HCC were randomized 1:1:1 to receive nivolumab 1 mg/kg plus ipilimumab 3 mg/kg administered every 3 weeks, followed by nivolumab maintenance (arm A), nivolumab 3 mg/kg plus ipilimumab 1 mg/kg followed by nivolumab maintenance administered every 3 weeks (arm B), or nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (arm C). Patients in arm A had higher in objective response rate (32% vs. 27% vs. 29% in arms A, B, and C, respectively) and median OS (22.8 vs. 12.5 vs. 12.7 months); however, this study was not powered to detect differences between treatment arms. Arm A did have higher rate of grade 3 or 4 treatment-related adverse events, and discontinuation of the study drug due to toxic effects. This combination regimen (treatment arm A) was subsequently given accelerated approval by the FDA to treat patients with HCC after progression on sorafenib. This combination is now under investigation as first-line therapy for patients with HCC (NCT04039607). The major trials evaluating ICI alone or as combination therapy in HCC are summarized in Table 2.

Several TKI and ICIs have been approved in the first and subsequent line settings for the management of advanced HCC. A general treatment algorithm is shown in Figure 2. In evaluating the optimal first line regimen for each patient, toxicity profiles of the drug in combination with the patient’s medical history and performance status should be taken into consideration, given the lack of randomized data comparing each approved regimen in the treatment-naïve setting. For example, a patient with significant cardiac co-morbidities, bleeding diathesis, and/or history of grade 3 varices with bleeding, may not be a candidate for anti-angiogenic treatment, and thus combination durvalumab and tremelimumab may be considered. Another patient with refractory autoimmune disease would not be a candidate for combination ICI, and thus TKI may be considered. Thus, the treatment of choice is often dependent on clinical factors while weighing the risks and benefits of each regimen for each individual patient.

Ongoing evaluations of combination strategies

ICI plus VEGF blockade
Enhancement of CD8+ T cell function with anti-angiogenic agents has been demonstrated in solid malignancies including HCC and renal cell carcinoma. VEGF pathway signaling has been implicated to diminish anti-tumoral immunity by several mechanisms, including reducing the cytotoxic ac-
<table>
<thead>
<tr>
<th>Trial (reference)</th>
<th>Sample size (n)</th>
<th>Inclusion criteria</th>
<th>Phase and comparator</th>
<th>Primary endpoint(s)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEYNOTE-224&lt;sup&gt;20&lt;/sup&gt;</td>
<td>104</td>
<td>Patients with BCLC stage B or C HCC who were intolerant to or progressed on sorafenib, that was not amenable to or refractory to curative treatment approach, with ECOG 0–1, and Child-Pugh class A liver function</td>
<td>2</td>
<td>ORR per RECIST v1.1&lt;sup&gt;27&lt;/sup&gt;</td>
<td>ORR 18% (95% CI 11–26%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pembrolizumab 200 mg every 3 weeks for up to 35 cycles</td>
<td></td>
<td>CR 1%, PR 16%, SD 44%</td>
</tr>
<tr>
<td>CheckMate 040&lt;sup&gt;19&lt;/sup&gt;</td>
<td>262</td>
<td>Advanced HCC who progressed on at least one prior line of treatment including sorafenib, with Child-Pugh B7 or A and ECOG 0–1</td>
<td>I/II</td>
<td>ORR (dose-expansion phase)</td>
<td>ORR 20% (95% CI 15–26%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nivolumab every 2 weeks</td>
<td></td>
<td>CR 1%, PR 18%, SD 45%</td>
</tr>
<tr>
<td>CheckMate 040&lt;sup&gt;26&lt;/sup&gt;</td>
<td>148</td>
<td>HCC not eligible for curative treatment with Child-Pugh class A liver function, ECOG PS 0–1</td>
<td>3</td>
<td>Safety and tolerability, and ORR</td>
<td>ORR 32% (arm A), 27% (arm B), and 29% (arm C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A: Nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for 4 doses</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B: nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C: nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CheckMate 459&lt;sup&gt;27&lt;/sup&gt;</td>
<td>743</td>
<td>Advanced HCC not eligible for locoregional therapies, Child-Pugh class A liver function, ECOG 0–1, with no prior systemic therapy</td>
<td>3</td>
<td>OS</td>
<td>Median OS 16.4 vs. 14.7 months (HR 0.85; P=0.075)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nivolumab 240 mg every 2 weeks or sorafenib 400 mg twice daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CheckMate 240&lt;sup&gt;22&lt;/sup&gt;</td>
<td>413</td>
<td>HCC with progression or intolerance to sorafenib treatment, BCLC stage B or C disease, Child-Pugh class A liver disease, ECOG 0–1</td>
<td>3</td>
<td>OS</td>
<td>Median OS 13.9 vs. 10.6 months (HR 0.781; P=0.0238)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pembrolizumab 200 mg every 3 weeks or placebo for up to 35 cycles</td>
<td></td>
<td>Median PFS 3.0 vs. 4.1 months (HR 0.775; P=0.0186)</td>
</tr>
<tr>
<td>IMBrave150&lt;sup&gt;13, 24&lt;/sup&gt;</td>
<td>336</td>
<td>Locally advanced, metastatic, or unresectable HCC with no prior systemic therapy, that was not amenable to curative or locoregional therapies or had progressed thereafter, with Child-Pugh liver function A and ECOG 0–1</td>
<td>3</td>
<td>OS</td>
<td>Median OS 19.2 vs. 13.4 months (HR 0.66; P=0.0009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atezolizumab 1,200 mg plus bevacizumab 15 mg/kg every 3 weeks or sorafenib 400 mg twice daily</td>
<td></td>
<td>Median PFS 6.8 vs. 4.3 months (HR 0.59; P&lt;0.001)</td>
</tr>
</tbody>
</table>
tivity of peripheral T cells, enhancing T regulatory cell activation, and inducing myeloid derived suppressor cells, which in turn elicit immunosuppressive effects by lymphocyte depletion, generation of oxidative stress, interfering with lymphocyte trafficking and activation of T regulatory cells. VEGF-A also directly induces FASL expression leading to apoptosis of CD8⁺ T cells. In preclinical models of HCC, anti-PD1 in combination with anti-VEGFR2 antibodies showed enhanced M1 and decreased M2 tumor-associated macrophages, as well as increased level of infiltrating CD8⁺ T cells.

With strong preclinical rationale, ICIs in combination with anti-VEGF TKIs have been evaluated in clinical trials but have shown mixed results. In a randomized double-blind phase III study, lenvatinib plus pembrolizumab was compared to lenvatinib alone in treatment-naïve HCC, where the primary endpoints of OS and PFS did not meet pre-specified statistical significance (median OS 21.2 vs. 19.0 months, HR 0.84, P=0.02; median PFS 8.2 vs. 8.0 months, HR 0.876, P=0.05). Similarly, another study evaluated the combination of cabozantinib plus atezolizumab compared to sorafenib in the first-line setting for patients with unresectable or advanced HCC, where the median PFS was improved in the combination arm (6.8 vs. 4.2 months, HR=0.63, P=0.0012), but there was no difference in survival (median OS 15.4 vs. 15.5 months, HR 0.9, P=0.44).

Conversely, the phase III ORIENT-32 trial evaluating PD-1 inhibitor sintilimab with IBI305 (bevacizumab biosimilar) improved OS as compared to sorafenib in patients with untreated hepatitis B virus associated HCC in an exclusively Chinese population (median OS NR vs. 10.4 months, HR 0.57, P<0.0001). In another phase III trial of PD-1 inhibitor camrelizumab combined with rivoceranib versus sorafenib alone, OS and PFS were superior in the combination arm (median OS 22.1 months vs. 15.2 months, HR 0.62, P<0.001; median PFS 5.6 vs. 3.7 months, HR 0.52, P<0.0001). This represents the longest OS observed to date in phase III trials involving patients with advanced HCC. Further research is needed to understand the role of ICI with VEGF TKI combination in the treatment of advanced HCC.

**ICI plus locoregional treatment**

It is hypothesized that combining ICIs with other treatment modalities (e.g., surgical resection, ablation, transarterial therapies, radiotherapy, etc.) can potentially have an improve
overall effect in patients with advanced HCC. Radiotherapy (RT) has been shown to enhance immunotherapeutic effects in various cancers. Radiation can prime the immune system by enhancing antigen presentation, promoting infiltration of cytotoxic T cells, and reprogramming the tumor microenvironment against the immune evasion of cancer. In preclinical models, liver-directed radiotherapy eliminates immunosuppressive hepatic macrophages, increases hepatic T cell survival and reduces hepatic siphoning of T cells. In HCC, preclinical data have shown combination of RT and ICIs to exhibit therapeutic synergism, superior tumor control, and improved OS. Despite encouraging preclinical findings, there are a small number of published prospective trials on combination of RT and ICI in HCC. Small series have shown promising clinical activity. In a propensity score matching analysis of approximately 64 patients with unresectable or recurrent locally advanced HCC who received Stereotactic Body Radiation Therapy in combination with ICI (SBRT-ICI) versus TACE at a single institution, the authors reported a significantly improved objective response rate (87.5% vs. 16.7%), 24-month PFS (77.8% vs. 2.1%) and 24-month OS (80.4% vs. 8.3%) in the SBRT-ICI arm. In a phase I multicenter trial, 14 patients with advanced or unresectable HCC received SBRT (40 Gy in 5 fractions) followed by either nivolumab alone or nivolumab in combination with ipilimumab. Clinical outcomes favored the combination ICI group, with overall response rate of 57% vs. 0%, median PFS of 11.6 months vs. 2.7 months, and median OS of 41.6 months vs. 4.7 months. The study was stopped due to slow accrual. Several ongoing studies are currently evaluating the efficacy of combination of ICI with SBRT in the neoadjuvant setting for early-stage HCC (NCT04857684), and in the first or subsequent line settings for advanced HCC (NCT05488522, NCT04913480).

SBRT has also been studied in combination with sorafenib. In the phase III NRG/RTOG 1112 trial, patients with advanced HCC were treated with sorafenib monotherapy or sorafenib with SBRT. The median OS was 12.3 vs. 15.8 months favoring the combination arm (HR 0.77; P=0.055). The median PFS was 5.5 vs. 9.2 months (HR 0.55; P=0.0001). There was no significant increase of grade 3 or higher adverse events in the combination arm.

Local ablation increases liver immunogenicity and activation of antigen presenting dendritic cells in HCC. In preclinical models, ablation increases T cell infiltration and immune checkpoint expression within and beyond the treatment zone, suggesting that the addition of ICI to ablation may re-

---

**Figure 2.** Approach to systemic treatment of advanced HCC. HCC, hepatocellular carcinoma; AFP, alpha fetoprotein.
result in synergistic antitumor activity. In a study of patients with advanced HCC treated with combination of CTLA-4 inhibitor tremelimumab and tumor ablation (radiofrequency ablation or chemoablation), authors reported a 26.3% tumor response rate and a median time to tumor progression of 7.4 months.

Intra-arterial therapies such as TACE and transarterial radioembolization (TARE) with Y90 have been widely adopted over the last two decades and are currently considered fairly standard treatment options in management of intermediate stage HCC in most high volume centers. There are preclinical data to suggest that TACE can improve liver immunogenicity and enhance ICI efficacy. In a cohort of patients with HCC treated with TACE, expression of immune checkpoints PD-1 and PD-L1 on tumor cells increased after treatment with TACE. While PD-L1 has not been shown to be a clinical marker of response to ICI in HCC as in other tumors, the upregulation of immune checkpoints suggests that TACE may induce an immunogenic tumor microenvironment. In a cohort of 34 patients treated with camrelizumab and TACE, similar changes were seen within the hepatic tumor microenvironment, where an enhanced number of released tumor antigens leads to local immune activation with infiltration of CD8+ T cells and natural killer cells. Furthermore, in patients with HCC treated with TARE, similar changes were seen within the hepatic tumor microenvironment, where an enhanced number of released tumor antigens leads to local immune activation with infiltration of CD8+ T cells and natural killer cells. These data suggest that the combining TACE with lenvatinib may be considered for patients with advanced HCC.

Intra-arterial therapies have also been studied in combination with sorafenib. In the multicenter phase II SORAMIC trial, patients with advanced HCC received sorafenib either alone or in combination with radioembolization. Patients who received combination therapy had higher objective response rate (61.6% vs. 29.8%, P<0.001), complete response rate (13.7% vs. 3.8%, P=0.022), median PFS (8.9 vs. 5.4 months, P=0.022), and hepatic median PFS (9.0 vs. 5.7 months, P=0.014). However, an improvement in OS was not seen in the combination arm. Similarly, the TACTICS trial evaluated TACE compared to TACE plus sorafenib in patients with unresectable HCC. Here, median PFS was significantly longer in the TACE plus sorafenib arm (25.2 vs. 13.5 months, P=0.006). One-year OS in the combination group was also prolonged (96.2% vs. 82.7%).

While combining locoregional and systemic therapies has been suggested as a way to enhance efficacy and tumor response rates in treatment of HCC, the ideal strategy has not yet been delineated and remains under investigation.

**SYSTEMIC THERAPIES IN NEOADJUVANT AND ADJUVANT SETTINGS**

Systemic therapies are now being evaluated in the adjuvant and neoadjuvant settings for early-stage HCC to improve the chance for cure. In a phase IIb study, 12 of 15 patients with unresectable HCC who were treated with neoadjuvant cabozantinib and nivolumab underwent successful margin negative resection. Furthermore, 5 of the 12 resected demonstrated major response on final pathologic evaluation.
in effector T cells and a distinct spatial arrangement of B cells in responders, suggesting the possibility of durable immunologic memory postoperatively conferred by pre-operative immune priming. Several ongoing randomized phase III trials are evaluating whether adjuvant ICI may reduce the risk of recurrence after curative-intent resection (KEYNOTE-937 evaluating pembrolizumab vs. placebo after curative-intent surgical resection or ablation,\(^6\)) CA209-9DX evaluating nivolumab vs. placebo for tumors at high risk of recurrence after curative-intent surgical resection,\(^6\) and IMbrave050 evaluating atezolizumab-bevacizumab vs. active surveillance after resection\(^6\)). At the time of interim analysis with a median follow up of 17.4 months, the primary endpoint (recurrence-free survival [RFS]) was met with HR of 0.72 (P=0.012), making atezo-bev the first adjuvant regimen to demonstrate a statistically significant and clinically meaningful improvement in RFS in patients with high risk of disease recurrence following local curative treatment.\(^6\) Whether this will translate to improving the cure rate and OS in this patient population remains in question. Given the possibility of inducing durable immune responses, ICI will likely begin to play a larger role in the treatment of early-stage HCC. Data from the aforementioned and several other ongoing trials will shed light on the ideal adjuvant and/or neoadjuvant strategies for these patients.\(^6\)

Management of HCC in special populations

An area of unmet need in the current understanding of HCC management is the treatment of patients with HCC who have concurrent comorbidities and medical conditions such as advanced cirrhosis (Child Pugh B), history of prior liver transplant, history of immunosuppressive conditions such as HIV infection. While the majority of clinical trials which have led to the approval of systemic agents for HCC have incorporated patients with Child Pugh A cirrhosis only, a significant portion of patients who present with HCC in clinical practice may have advanced cirrhosis. Real-world analyses have sought to answer the question of efficacy of current systemic therapies in patients with advanced cirrhosis. In a retrospective real-world study of 216 patients with HCC who were treated with atezo-bev, 24% were noted to have Child Pugh B cirrhosis. The median OS was significantly longer in the Child Pugh A group (16.8 months) compared to the Child Pugh B group (6.7 months; P=0.0003).\(^5\) PFS was also longer in the Child Pugh A group (7.6 vs. 3.4 months). However, treatment related adverse events were noted to be similar in both groups.\(^6\) Although more patients with Child Pugh B disease experience grade ≥3 bleeding events and atezolizumab related adverse events compared to the Child Pugh A group (10% vs. 4%; 15% vs. 4%), grade ≥3 atezolizumab-related hepatitis only occurred in patients with Child Pugh A disease (8%). Discontinuation of treatment because of treatment-related adverse events was 11% among all patients, suggesting that atezo-bev may be tolerable for patients with Child Pugh A or B cirrhosis. However, the limited number of patients in this study warrants a larger prospective study to investigate safety of atezo-bev in patients with Child Pugh B disease. Further, nivolumab has been evaluated in a phase I/II open-label multicenter trial in patients with advanced HCC and Child Pugh B cirrhosis. In 24 sorafenib-treated patients and 25 sorafenib-naïve patients, the objective response rate was 12%. Treatment-related adverse events were reported in 51% of patients, leading to treatment discontinuation in 2% of patients, where safety was comparable to that reported for Child Pugh A patients. Given the limited data to guide in the treatment of patients with advanced HCC and Child Pugh B disease, the recommendation for each individual patient may be variable and dependent on several considerations. For example, those with Child Pugh B7 may be more likely to benefit from treatment than B8 or B9. The etiology of liver dysfunction (i.e., cirrhosis versus tumor burden) may be helpful in understanding whether patients with Child Pugh B8 or B9 may benefit. Furthermore, other markers of liver function including albumin-bilirubin grade and the Model for End-Stage Liver Disease score may be used to stratify patients with cirrhosis.

Up to 10–15% of liver transplant recipients may experience HCC recurrence. TKIs have been evaluated retrospectively in the post-transplant patient population. In a systematic review and meta-analysis of eight studies, median OS of 12 months with acceptable safety profile was reported with sorafenib. In a multi-center retrospective study of 28 post-transplant patients with HCC, regorafenib was evaluated in patients who progressed on sorafenib, with median OS of 12.9 months following treatment initiation.\(^6\) There is a paucity of data examining the efficacy and safety of ICI in the post-transplant setting.\(^6\) In a retrospective pilot evaluation to assess the safety and efficacy of ICI in patients post liver transplant, 7 patients with metastatic cancer with a history of
liver transplant were treated with ICI for either HCC or melanoma. 2 of 7 patients developed rejection within a median time of 24 days. 1 patient achieved a complete response, 3 patients had progression of disease, and 3 patients discontinued therapy prior to restaging assessments. Clinical trials are underway evaluating safety and efficacy of ICI in post-transplant HCC, and at this time generally are not given outside of a clinical trial.

CONCLUSIONS

The treatment landscape for advanced HCC has transformed over the last decade. ICIs are now the backbone of most treatment strategies in clinical practice for advanced HCC and continue to be investigated in clinical research in novel combinatorial strategies. Despite these major advances, many challenges still exist in the management of patients with advanced HCC. One such challenge frequently faced in the clinic is the appropriate management of patients with advanced cirrhosis, given that most of the currently approved treatments were studied in patients with and are approved in patients with Child Pugh A cirrhosis. Frequently, patients with HCC tend to be more debilitated from their illness and have more complications from their underlying cirrhosis, than those represented in major clinical trials, and thus a gap still exists in finding the optimal treatment for these patients. Secondly, the optimal sequencing of systemic therapies remains unknown, particularly as it relates to the two ICI-based combination treatments now approved in the first-line setting (atezo-bev and durva-treme). It is also poorly understood whether combination ICI strategies can be effective after progression on PD-1 monotherapy, and whether ICI strategies can improve outcomes when given in the peri-operative setting for patients with early-stage HCC. The data from several ongoing clinical trials will shed light on the optimal combination strategies in these settings. Finally, the identification of biomarkers to assess response and development of resistance to ICIs is crucial and is a significant area of ongoing research. The incorporation of systemic therapy in the management of early-stage and intermediate-stage HCC, and further advances in effective combination strategies for advanced HCC are foreseen in the near future.

Authors’ contribution

Conception and design: KS, JDY. Administrative support: N/A. Provisions of study materials or patients: N/A. Collection and assembly of data: N/A. Data analysis and interpretation: N/A. Manuscript writing: all authors. Final approval of the manuscript: all authors.

Acknowledgements

This work was supported by R01 HL149946, R01 CA273925 grants, Cedars-Sinai Cancer and Cancer Biology Program discovery fund to EKK. EKK also received research funding from Surface Oncology Inc to investigate IL-27 blockade in HCC. JDY’s research is supported by American College of Gastroenterology Junior Faculty Development Award, Department of Defense Peer Reviewed Cancer Research Program Career Development Award (CA191051) and the National Institutes of Health (K08CA259534).

Conflicts of Interest

JG has served as a consultant/advisory role for Amgen, Astellas Pharma, QED Therapeutics, Exelixis, Elsevier, EMD Serono/Merck, Eisai, Pfizer/Myovant, Bayer, Basilea, HalioDx, Natera, Incyte, AVEO, Janssen Biotech, Seagen, MJH Life Sciences. AEH has served as a consultant for Varian, Genentech, Merck, BMS, Abbvie, Valar and Farady. JDY provides a consulting service for AstraZeneca, Eisai, Exact Sciences, Exelixis, Fujifilm Medical Sciences, and Gilead Sciences.

REFERENCES

5. CT/MRI LI-RADS® v2017 CORE. [Internet]. American College of


Taiwan Association for the Study of the Liver-Taiwan Society of Cardiology Taiwan position statement for the management of metabolic dysfunction-associated fatty liver disease and cardiovascular diseases

INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) and nonalcoholic fatty liver disease (NAFLD) are significant global health issues. In the general population, the incidence of MAFLD ranges from 15% to 30%. The prevalence of NAFLD is approximately 55% in patients with type 2 diabetes mellitus (T2DM) and up to 80% in those with obesity. The incidence rates of T2DM, hypertension, low high-density lipoprotein cholesterol levels, and hypertriglyceridemia are 9%, 8.4%, 9.6%, and 23.6%, respectively, in patients with biopsy-proven NAFLD. The prognosis of hepatic outcomes in patients with MAFLD is associated with the severity of liver fibrosis. Studies have demonstrated a significantly higher incidence of cirrhosis, hepatocellular carcinoma (HCC), and liver-related death in patients with NAFLD and fibrosis. A study revealed an increase in cardiovascular events in patients with MAFLD. The latest international consensus statements on the association between MAFLD and the risk of cardiovascular disease (CVD), which have been developed by experts from six continents, indicate that patients with MAFLD have higher cardiovascular events and mortality than individuals without MAFLD. In addition, CVD is the leading cause of death in patients with MAFLD.

Keywords: MAFLD; Cardiovascular disease; Position statement; Taiwan

Abbreviations:
MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; HCC, hepatocellular carcinoma; CVD, cardiovascular diseases; IR, insulin resistance; NASH, nonalcoholic steatohepatitis; CAD, coronary arterial disease; VTE, venous thromboembolism; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MRE, magnetic resonance elastography; FIB-4, fibrosis-4; LSM, liver stiffness measurement; MI, myocardial infarction; HF, heart failure; HfPEF, HF with preserved ejection fraction; LV, left ventricular; AF, atrial fibrillation; EASL, European Association for the Study of the Liver; APASL, Asian Pacific Association for the Study of the Liver; PPAR-γ, peroxisome proliferator-activated receptor gamma; GBMT, endoscopic bariatric and metabolic therapies; GLP-1RA, glucagon-like peptide-1 receptor agonist; DPP-4i, dipeptidyl peptidase-4 inhibitor; SGLT-2i, sodium-dependent glucose cotransporter-2 inhibitor; OCA, obeticholic acid; CHB, chronic hepatitis B; CHC, chronic hepatitis C

Received: Aug. 19, 2023 / Revised: Sep. 22, 2023 / Accepted: Sep. 25, 2023
MAFLD. Metabolic comorbidities are the leading risk factors for cardiovascular events and liver-related mortality in patients with MAFLD. T2DM intensifies the risks of CVD and chronic kidney disease due to increased insulin resistance (IR). The incidence of T2DM and hypertension also increases with the severity of MAFLD. A meta-analysis revealed that T2DM, low high-density lipoprotein cholesterol levels, hypertriglyceridemia, and hypertension are significantly associated with a high risk of severe liver diseases, including cirrhosis, HCC, and liver-related mortality.

**Position statement 1:** MAFLD can lead to hepatic and extrahepatic morbidity and mortality.

**Definition and diagnosis of MAFLD**

In 2020, the international expert consensus recommended changing the term NAFLD to MAFLD. Compared with NAFLD, MAFLD adequately reflects similar pathophysiological mechanisms and cardiometabolic risk factors for fatty liver disease and CVDs, such as metabolic dysfunction, obesity, IR, and dyslipidemia. MAFLD is diagnosed based on histological, imaging, or biomarker evidence of hepatic steatosis in patients with overweight/obesity, T2DM, or at least two metabolic risk factors (Fig. 1).

**Diagnostic tools**

Liver biopsy remains the gold standard for the diagnosis and assessment of histological features in patients with NAFLD. However, the invasiveness of liver biopsy limits its routine use in clinical settings. Ultrasound-based modalities are widely adopted as the first-line screening tools for hepatic steatosis; they have excellent performance for detecting moderate and severe steatosis, with a sensitivity and specificity of 84.8% (95% confidence interval [CI]: 79.5–88.9%) and 93.6% (95% CI: 87.2–97.0%), respectively. Ultrasound-based transient elastography enables the quantitative evaluation of liver stiffness and steatosis. The area under the receiver operative characteristic curve of the ultrasonic controlled attenuation parameter for the detection of steatosis reached 0.95 in a previous study. Magnetic resonance imaging-derived proton density fat fraction is the most sensitive noninvasive method for quantifying hepatic steatosis, with an area under the receiver operative characteristic curve of 0.95. Several noninvasive serum biomarkers, including the fatty liver in-

---

**Figure 1.** Definition of metabolic dysfunction-associated fatty liver disease. BMI, body mass index; HDL, high density lipoprotein.
dex, hepatic steatosis index, NAFLD liver fat score, and lipid accumulation product can be used to evaluate hepatic steatosis with moderate-to-good diagnostic performance (sensitivity: 86–93%, specificity: 40–71%).

Position statement 2: MAFLD is defined as the presence of hepatic steatosis plus metabolic derangements.
Position statement 3: Abdominal ultrasonography is a useful and convenient tool for identifying hepatic steatosis.

**MAFLD pathogenesis and risks**

In 1998, the two-hit theory was proposed for the pathogenesis of NAFLD; it involves increased fat accumulation and the inflammatory cascade in the liver. IR in the adipose tissue, muscle, and liver is a key factor in the first hit. It is associated with energy imbalance caused by excessive caloric intake. Hepatic steatosis is caused by an imbalance between hepatic lipid storage and clearance, leading to excessive triglyceride-rich droplets in hepatocytes. In the second hit, the inflammatory cascade is overly activated by inflammatory cytokines, adipokines, lipotoxicity, endoplasmic reticulum stress, oxidative stress, and mitochondrial dysfunction. Unresolved hepatic steatosis can progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and even HCC in severe cases. Recent research has identified genetic factors, epigenetics, and gut microbiota dysbiosis as other MAFLD-associated molecular and metabolic elements, resulting in the “multiple-hit” pathomechanism.

Figure 2 presents the pathophysiological interaction between MAFLD and CVD. The “multiple hits” involved in the pathogenesis of MAFLD converge to a vicious cycle that promotes the development and progression of atherosclerosis and CVD. In patients with MAFLD, the severity of hepatic steatosis and fibrosis is correlated with the coronary atheroma burden and atherosclerosis. Moreover, inflammation and IR in MAFLD may increase the platelet count and the number of coagulation factors, which are associated with coronary arterial disease (CAD) and venous thromboembolism (VTE).

Metabolic disorders and genetic origins are involved in the development of MAFLD and CVD. Multiple hits resulting from the interactions between genetic and environmental risk factors for MAFLD and CVD contribute to the occurrence...

---

**Figure 2.** Pathophysiological mechanisms underlying the interaction between MAFLD and CVD. MAFLD, metabolic associated fatty liver disease; CVD, cardiovascular disease.
Lifestyle factors
In genetically susceptible individuals, a sedentary lifestyle, a high sugar/saturated fat diet, metabolic derangements, and gut dysbiosis lead to MAFLD development and its progression. Lifestyle changes, including limited intake of dietary fructose, are highly recommended.

Metabolic factors
Risk factors for MAFLD include male sex, advancing age, obesity, IR, T2DM, and hyperlipidemia, which are linked to gut dysbiosis. IR is significantly involved in the pathogenesis of MAFLD and its progression to NASH, with T2DM being strongly associated with MAFLD, NASH, and CVD. Cholecystectomy is an independent risk factor of MAFLD, which is attributable to altered bile acid enterohepatic circulation.

Genetic factors
Several genetic variants (PNPLA3, TM6SF2, and MBOAT7) can increase the susceptibility to NAFLD. However, a Mendelian randomization analysis revealed no causal relationship between the NAFLD-associated PNPLA3 variant and CVD. Among the NAFLD-related genetic variants, TM6SF2 appears to be protective against VTE, whereas MBOAT7 may exert unfavorable effects.

Others
Other risk factors include steatogenic drugs, male sex, and infections. Coronavirus disease 2019; hepatitis C; acquired immunodeficiency syndrome; Helicobacter pylori-induced peptic ulcers; and periodontitis caused by Bacteroidetes, Candidatus Saccharibacteria, Firmicutes, and Proteobacteria worsen MAFLD.

Position statement 4: MAFLD and CVD have similar risk factors that exacerbate their progression. Identifying these risk factors is crucial for effective management and treatment.

Screening strategy for MAFLD in patients with CVD
Who should be screened?
Patients with MAFLD who have T2DM, central obesity, a sedentary lifestyle, and metabolic syndrome have a high risk of advanced fibrosis. Moreover, the severity of fibrosis is associated with cardiovascular risk in patients with steatosis or steatohepatitis. Thus, MAFLD surveillance should be consid-
Position statement 5: MAFLD should be considered in patients with CVD, irrespective of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Screening procedure
The screening tool should effectively identify patients with MAFLD who have advanced liver fibrosis. Transient elastography is more cost-effective than magnetic resonance elastography (MRE) for detecting advanced liver fibrosis, although its sensitivity and specificity are compromised. Thus, in patients suspected of having advanced fibrosis or those with inconclusive sonography and transient elastography findings, MRE should be considered. Indirect serological biomarkers include AST levels, AST-to-platelet ratio, fibrosis-4 (FIB-4) score, NAFLD fibrosis score, and AST-to-ALT ratio. Direct serological biomarkers include the enhanced liver fibrosis test score and FibroMeter NAFLD test score.

Fibrosis assessment
Fibrosis assessment is crucial in patients with MAFLD. Primary care practitioners, gastroenterologists, cardiologists, and neurologists should screen for advanced fibrosis in patients with MAFLD and CVD. The FIB-4 index may be practical, as the calculation is straightforward and is based on widely available, simple, and cost-effective tests. As no single measurement or threshold value has high sensitivity and specificity (≥80%), a sequential algorithm having the FIB-4 index as the first-line test and liver stiffness measurement (LSM) as the second-line assessment is recommended. Figure 4 presents the algorithm recommended for MAFLD screening and liver fibrosis assessment among patients with CVD. The recommended algorithm is based on both clinical evidence and expert consensus. A meta-analysis revealed that a sequential combination of FIB-4 scores of <1.3 and ≥2.67 and subsequent LSM scores of <8.0 and ≥10.0 kPa could rule-in and rule-out advanced fibrosis, with a sensitivity of 66% (95%}

Figure 4. Algorithm for MAFLD screening and fibrosis assessment among CVD patients. MAFLD, metabolic associated fatty liver disease; CVD, cardiovascular disease; FIB-4, fibrosis-4.
MAFLD is associated with CAD progression. MAFLD is also associated with worsened outcomes in patients undergoing coronary artery bypass grafting and percutaneous coronary angioplasty. In patients with myocardial infarction (MI), concomitant MAFLD exacerbates the risk of cardiovascular events and death. A large biobank analysis reported the association of MAFLD with cardiovascular and all-cause mortality. Patients with both non–ST-segment elevation MI and MAFLD have a high risk of premature ventricular complexes and ventricular tachycardia.

**MAFLD and arterial hypertension**

High blood pressure may predict MAFLD onset independently of conventional risk factors. A recent study in Taiwan revealed that patients with fatty liver have a high risk of prevalent and incident hypertension and/or diabetes. Moreover, the risk increases with an increase in the severity of fatty liver. Another study suggested that effective hypertension control reduces the risk of MAFLD.

**MAFLD and heart failure**

In patients with heart failure (HF) with preserved ejection fraction (HFrEF), the prevalence of MAFLD is approximately 50%. Patients with MAFLD have high left ventricular (LV) filling pressure in addition to a more fibrotic LV myocardium and worse global longitudinal strain. Increased hepatic sinusoid resistance and venous return impairment can lead to a high normal cardiac output and high LV mass, which is characteristic of obstructive HFrEF. MAFLD may affect cardiac metabolism, and fibrosis may promote the formation of spontaneous portosystemic shunts, altering arterial blood flow and systemic vascular resistance in patients with HFrEF, which are associated with cirrhosis and advanced liver disease.

**MAFLD and cardiac arrhythmias**

The incidence of QT interval prolongation is high in patients with MAFLD and T2DM. Ventricular arrhythmias, atrioventricular blocks, and atrial fibrillation (AF) are more frequent in patients with MAFLD. After catheter ablation, liver fibrosis is linked to adverse atrial remodeling and recurrent AF in patients with MAFLD. The Rotterdam study reported an association between AF and liver stiffness, but not steatosis. The conflicting results may be attributed to heterogeneous patient backgrounds.
MAFLD and thromboembolic diseases
MAFLD is an independent risk factor for VTE, and 81% of patients with VTE have MAFLD. The levels or activities of von Willebrand factor; factors VII–IX, XI, and XII; and plasminogen activator inhibitor-1 are high in patients with MAFLD. Patients with NASH have higher anticardiolipin immunoglobulin G levels than those with MAFLD, suggesting the association of thrombotic risks with liver fibrosis. Obesity is a VTE-associated risk factor in MAFLD. However, the potential benefits of different interventions, such as body-weight reduction, aerobic exercise, bariatric surgery, and anticoagulation medications, for VTE risk warrant further investigation.

Position statement 7: MAFLD increases the risks of hepatic-related and cardiovascular events, and it increases the risk of CVD.

Screening and management strategy of cardiovascular risks in patients with MAFLD
In patients with MAFLD, CVD risk screening and early management are recommended. A regional, validated risk calculator can be used to stratify the 10-year ASCVD or CAD risk in these patients. In patients with a high risk of CAD or angina, stress or imaging tests for CAD should be considered. If risk factors, such as hypertension, obesity, T2DM, and advanced age, are present, referral for echocardiography and natriuretic peptide testing should be considered in symptomatic cases. Early referral to a cardiologist is highly recommended for symptomatic cases or patients with MAFLD who have high cardiovascular risk.

Position statement 8: In patients with MAFLD, cardiovascular risk screening and management are recommended. In symptomatic or high-risk cases, referral and multidisciplinary care involving cardiologists are highly recommended.

Linking care of MAFLD and CVD: Decreasing risks of CVD and liver cancer/HCC

Nonpharmacological management of MAFLD/NAFLD

Lifestyle modification
Lifestyle interventions that reduce bodyweight are crucial for managing NAFLD. Approximately 5% weight loss is required to improve liver steatosis, and >10% weight loss is required for managing both liver steatosis and fibrosis. However, sustained weight loss is challenging. Approximately 21.2% of patients with initial weight loss regained weight after a median follow-up of 32.3 months. Thus, a multidisciplinary approach involving physicians, psychologists, behavioral therapists, dieticians/nutritionists, patients’ families, patient support groups, and digital support is pivotal for lifestyle interventions.

Dietary control
Excessive dietary intake of calories, saturated fats, refined carbohydrates, and sugar-sweetened beverages is common in patients with NAFLD and obesity. Dietary macronutrients are involved in the pathogenesis of NAFLD. For instance, fructose promotes hepatic steatosis and inflammatory signaling, and polyunsaturated fatty acids exhibit anti-inflammatory effects. The current guidelines of the European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL) recommend a hypocaloric diet (500–1,000 kcal deficit). Several trials support changing the amount and type of dietary carbohydrate/fat or adopting the Mediterranean diet, as both strategies can improve hepatic steatosis, regardless of weight loss. Furthermore, the Mediterranean diet is effective in primary CVD prevention. Regular coffee consumption is also associated with low risks of NAFLD and liver fibrosis.

Exercise
Exercise improves MALFD/NAFLD through various mechanisms, such as the upregulation of several signaling pathways, particularly those involving the peroxisome proliferator-activated receptor gamma (PPAR-γ). Exercise may downregulate mammalian target of rapamycin complex 1 signaling, further alleviating MAFLD/NAFLD. Exercise training is beneficial for hepatic and cardiometabolic function in patients with MAFLD/NAFLD. It improves vascular stiffness...
and endothelial dysfunction, thereby decreasing cardiovascular risk. By reducing fibrosis, vigorous exercise improves the histological findings of NASH. Regular and moderate exercise for at least 150 minutes per week or increasing activity levels for >60 minutes per week can ameliorate MAFLD/NAFLD.

Aerobic exercise, defined as continuous and rhythmic activities requiring the use of large muscle groups, is the primary training modality assessed in NAFLD exercise studies. By contrast, the benefit of resistance training remains controversial because of the heterogeneity of training intensity and protocols. A combination of aerobic and resistance training is expected to outperform either exercise modality. Alternative activities, such as yoga, Pilates, and tai chi, have exhibited beneficial effects in pilot studies. Updated guidelines of the AASLD and EASL strongly recommend any type of sustained individualized exercise for patients with MAFLD/NAFLD.

**Bariatric surgery**

Bariatric surgery leads to a sustained weight loss of up to 30% in patients with obesity, in addition to improving T2DM, NASH/NAFLD, morbidity, and mortality. Patients undergoing bariatric surgery showed NASH resolution and fibrosis regression 5 years postoperatively. Bariatric surgery also reduced CVD risk and CVD-associated morbidity in patients with obesity and NAFLD. In addition, endoscopic bariatric and metabolic therapies (EBMT) improved aminotransferase levels and decreased NAFLD activity scores in patients with obesity and NAFLD. However, well-designed prospective studies are warranted to assess the hepatic and cardiovascular benefits of EBMT in patients with NAFLD and obesity.

**Position statement 9:** Lifestyle modification constitutes the basic and important approach.

**Position statement 10:** Bodyweight reduction is the cornerstone of the nonpharmacological management of MAFLD; however, long-term bodyweight control remains a concern.

**Pharmacological intervention for MAFLD**

Although no drugs have been approved for MAFLD, the treatment of metabolic conditions closely associated with MAFLD may reverse IR, thereby ameliorating steatohepatitis and preventing fibrosis. Although lifestyle modification and weight loss are recommended as first-line interventions and can effectively reduce steatosis, inflammation, and fibrosis, they are often unsuccessful. Therefore, pharmacological therapy may address the gap in treatments inhibiting MAFLD progression. Table 1 summarizes the investigated drugs for MAFLD. The use of approved antidiabetic drugs, including biguanides, glucagon-like peptide-1 receptor agonists (GLP-1RAs), dipeptidyl peptidase-4 inhibitors (DPP-4is), sodium-dependent glucose cotransporter-2 inhibitors (SGLT-2is), and PPAR agonists, has been investigated in patients with NASH. Novel agents for NASH/NAFLD are in different phases of clinical development; their mechanisms of action include participation in de novo hepatic lipogenesis, mitochondrial fatty acid oxidation, inflammation, cell injury, collagen deposition, and fibrinolysis.

**Vitamin E**

Oxidative stress plays a key role in the pathogenesis of NASH; thus, vitamin E is justifiable as a therapeutic agent for NASH. Randomized controlled trials (RCTs) have been conducted in nondiabetic adults, children, and adolescents with biopsy-proven NASH. Pooled analyses have demonstrated that vitamin E significantly decreases aminotransferase levels and improves the histological characteristics of NASH, except for liver fibrosis. In an RCT involving patients with coexisting T2DM and NASH, 18 months of vitamin E supplementation histologically improved steatosis. However, the role of vitamin E in NASH and advanced fibrosis or cirrhosis remains inconclusive.

The safety concerns of vitamin E should be considered. In one study, all-cause mortality was high in patients taking a high dose (>800 IU/day) of vitamin E. Moreover, vitamin E increases the risk of HF in patients with vascular disease or T2DM and the risk of prostate cancer in healthy men. Although a high-vitamin E diet is associated with reduced stroke risk, it may significantly increase the risk of hemorrhagic stroke. In summary, vitamin E supplementation at a daily dose of 800 IU may be considered in nondiabetic adults with biopsy-proven NASH. The associated risks and benefits should be fully discussed with each patient before initiating therapy.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver effects</th>
<th>CV effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit. E</td>
<td>Improve steatosis, ballooning hepatocyte, and inflammation in non-T2DM patients; but not improve fibrosis</td>
<td>May increase risk of heart failure in T2DM patients</td>
</tr>
<tr>
<td>Obeticholic acid</td>
<td>Improved liver fibrosis without worsening NASH in patients with F2/F3 fibrosis. Safety concern</td>
<td>Little in changes the risk of cardiovascular event.</td>
</tr>
<tr>
<td>Statin</td>
<td>No benefits or harm</td>
<td>Prevent cardiovascular risk</td>
</tr>
<tr>
<td>Metformin</td>
<td>Not improve fibrosis</td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>Decrease content of hepatic fat and improve parameters of NASH in T2DM or non-T2DM</td>
<td>Reduces event of cardiovascular disease in T2DM and NASH</td>
</tr>
<tr>
<td>Glucagon-like peptide 1 receptor agonists</td>
<td>Effective of improving hepatic steatosis and liver enzymes for NAFLD patients. Efficacy in fibrosis regression needs study.</td>
<td>Beneficial effects on renal and cardiovascular complications in T2DM patients</td>
</tr>
<tr>
<td>Sodium-glucose cotransporter 2 inhibitors</td>
<td>Positive effects on hepatic steatosis in T2DM and NAFLD Role of regression of hepatic fibrosis needs investigation</td>
<td>Offer significant cardiometabolic and renal protection</td>
</tr>
<tr>
<td>Dipeptidyl peptidase IV (DPP-IV) inhibitors</td>
<td>Not reduce hepatic steatosis or fibrosis in overweight T2DM</td>
<td>Lowering cardiovascular diseases incidence in T2DM patients</td>
</tr>
</tbody>
</table>

CV, cardiovascular; MAFLD, metabolic dysfunction-associated fatty liver disease; Vit, vitamin; T2DM, type 2 diabetes mellitus; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease.
Bile acids
The AASLD or EASL does not recommend ursodeoxycholic acid, a natural dihydroxy bile acid, for the treatment of NAFLD or NASH because of insufficient evidence regarding its beneficial effects on liver histology.

Obeticholic acid (OCA) is an analog of the bile acid chenodeoxycholic acid and a potent farnesoid X receptor agonist. Although the primary endpoints were met in the phase 2 FLINT trial and phase III REGENERATE trial of OCA, the U.S. Food & Drug Administration (US FDA) raised safety concerns regarding pruritus, high low density lipoprotein (LDL) levels, and limited changes in cardiovascular risk. Consequently, the AASLD, EASL, and APASL do not recommend OCA for off-label use for the treatment of NASH by 155-157.

Lipid-lowering agents
Statins may decrease LDL levels and cardiovascular risk in patients with NAFLD and NASH without liver decompensation. However, according to the AASLD and EASL, this treatment does not benefit or harm patients with liver disease. Recent study has presented mixed findings regarding the role of PCSK9 inhibitors in managing early-stage NAFLD, emphasizing the need for extensive long-term research to ascertain their efficacy and safety. 158

Glucose-lowering agents
Metformin
Metformin is a biguanide with a mild insulin-sensitizing effect. It is traditionally the first-line therapy for T2DM. In patients with NAFLD unresponsive to lifestyle modifications, biochemical improvement was observed after metformin treatment. Hepatic fat reduction with weight loss was also noted in a proportion of patients with NASH who were treated with metformin. In an open-label trial, metformin in combination with rosiglitazone further improved liver histology in patients with NASH. However, a meta-analysis of metformin trials did not reveal improvements in the liver disease activity score or fibrosis stage. Overall, insufficient evidence supports the routine use of metformin in patients with NASH.

Pioglitazone
Pioglitazone was found to improve liver function, decrease hepatic fat, and improve NASH features in clinical trials and systemic reviews, regardless of the diabetic status. Although weight gain was observed after pioglitazone therapy, data on other thiazolidinediones are limited. In patients with T2DM and NASH, pioglitazone reduced CVD events.

GLP-1RAs
GLP-1RA is a new class of antidiabetic agents for T2DM that can improve weight loss, glycemic control, and liver enzyme levels by activating the gut-derived incretin pathway. GLP-1RAs exhibit beneficial renovascular and cardiovascular effects on T2DM. Histological findings of the phase 2 LEAN RCT revealed that patients with T2DM receiving liraglutide for 48 weeks had higher NASH resolution and lower fibrosis progression than those receiving placebo. In a phase 2 trial of semaglutide, compared with placebo, 72-week semaglutide treatment resulted in significantly higher NASH resolution in patients with biopsy-proven NASH and F1–F3 liver fibrosis. However, the semaglutide trial did not reveal beneficial effects in improving the fibrosis stage. In a systematic review and meta-analysis of patients with T2DM and NAFLD, GLP-1RAs effectively improved intrahepatic, visceral, and subcutaneous adipose tissue; liver function; body mass index; waist circumference; and glucose/lipid profiles but did not improve liver fibrosis markers, such as FIB-4 and NAS. The main adverse events were mild-to-moderate gastrointestinal discomfort, such as poor appetite, constipation, diarrhea, and hypoglycemia, which resolved within a few weeks. Although a few small-scale studies have reported that GLP-1RAs are associated with NASH resolution and fibrosis regression, more large-scale studies are warranted.

SGLT-2is
SGLT-2is are antidiabetic agents that have extended benefits, and they are approved for reducing adverse outcomes in nondiabetic patients with HF and chronic kidney disease.

An observational study revealed that add-on treatment with 50 mg ipragliflozin for 45 weeks improved glycemic control and normalized ALT levels in patients with T2DM and NAFLD who were unresponsive to incretin-based therapy. SGLT-2is also improved glycemic control and liver function in patients with T2DM and NAFLD and exclusively caused weight loss. The efficacy of canagliflozin, dapagliflozin, and empagliflozin for NAFLD or NASH has been investigated in RCTs involving patients with T2DM with or without NAFLD, and the hepatic benefits, including aminotransferase, steatosis, and fibrosis improvements, of SGLT-2is have been not-
ed. Overall, SGLT-2is have exhibited positive effects on hepatic steatosis in meta-analyses; however, their effect on liver fibrosis requires further investigation.

**DPP-4is**

DPP-4 inhibition reduces glucagon levels, delays gastric emptying, stimulates insulin release, and augments pancreatic beta-cell regeneration. DPP-4is may alleviate T2DM-related microvascular complications.

Early interventions with sitagliptin in patients with T2DM may have long-lasting reno- and islet-protective effects. However, whether sitagliptin increases the risk of hospitalization in patients with HF remains debatable. Sitagliptin decreased CVD incidence in patients with T2DM. However, 12-week sitagliptin therapy did not reduce hepatic steatosis or fibrosis in overweight patients with T2DM. Moreover, it did not reduce aminotransaminase levels in patients with NASH. Vildagliptin exhibited a CVD risk comparable to sitagliptin, and it prevented the progression of T2DM-related CVD by improving LDL heterogeneity.

**Position statement 11:** Regressing hepatic steatosis/fibrosis and improving cardiovascular/metabolic outcomes are the optimal goals of pharmacological intervention for MAFLD.

**MAFLD/CVD and other types of hepatitis**

The prevalence of coexisting MAFLD and chronic hepatitis B (CHB) or chronic hepatitis C (CHC) is 30–70%, and MAFLD occurs in 13.6–59.3% of patients with CHB. An inverse association has been reported between hepatitis B virus replication and hepatic steatosis, as fat deposition in hepatocytes and a related increasing inflammatory status may inhibit or suppress viral replication. By contrast, patients with MAFLD and CHB tend to experience accelerated liver disease progression and exhibit more liver-related complications. Furthermore, their death rate is higher than that of patients with CHB or MAFLD. More studies are warranted to explore the effect of coexisting CHB on CVD risk in patients with MAFLD.

Hepatic steatosis, a common histological feature, is detected in 30–70% of patients with CHC. The coexistence of CHC and MAFLD occurs in 9–38% of cases. Data suggest that metabolic disturbances are highly prevalent in patients with CHC, placing them at higher risks of CVD, carotid and coronary atherosclerosis, and myocardial dysfunction. Nevertheless, no direct evidence suggests that MAFLD aggravates CVD risk in patients with CHC.

Delineating the relative contributions of alcohol consumption in patients with MAFLD having metabolic risk factors is challenging. Alcohol consumption may deteriorate liver disease and may lead to CVD development in patients with MAFLD through an additive or synergistic mechanism.

**SUMMARY**

MAFLD has become an important health issue globally. Because of underlying IR or metabolic derangement, substantial cross-talk occurs between hepatic outcomes (steatosis, a hepatic manifestation of metabolic syndrome) and cardiovascular events (CVD, a cardiac manifestation). In this positional statement, 11 important clinical issues regarding the diagnosis, screening, and assessment of MAFLD; the importance of the co-management of MAFLD and CVD; and potential management strategies have been addressed and discussed by both hepatologists and cardiologists. The benefits of various lifestyle modifications and updates on different pharmacological interventions for CVD and steatosis-associated advanced fibrosis have also been briefly reviewed. We hope that these statements simplify the clinical practice of gastroenterologists/hepatologists and cardiologists for treating patients with MAFLD or CVD. These statements also aim to draw the attention of general practitioners to emerging MAFLD, and setting optimal goals for clinical management is crucial.

**Authors’ contribution**

Conceptualization, CJL, YWW, and PNC; Writing, review, and editing the Original Draft, PNC; Review & Editing, WJC and CJYH; Writing and review: CLL, MLC, CCW, WTC, CYW, CYL, CLH, CYP, MLY, THC, J.FH, YHH, CYC, CEC, HCL, YHL, THL, JHK, TDW, and PYL.

**Acknowledgements**

This work was supported by the National Science and Technology Council, Executive Yuen, Taiwan (MOST 109-2314-B-002 -091 -MY3; NSTC 112-2314-B-002 -205 -MY3).
This work was also partly supported by the “Center of Excellence for Metabolic Associated Fatty Liver Disease, National Sun Yat-sen University, Kaohsiung, Taiwan” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan, MOHW112-TDU-B-221-124007, NYCUKMU-111-I001 and NYCUKMU-111-I004, and by the Taiwan Association for the Study of the Liver.

Conflicts of Interest
Chern-En Chiang: I received honorarium from Astrazeneca, Bayer, Boehringer Ingelheim, Daiichi-Sankyo, Eli Lilly, Menarini, MSD, Novartis, Novo Nordisk, Pfizer, Sanofi, Viatris. The other authors declare no conflict of interests.

REFERENCES
2. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and non-alcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism 2019;92:82-97.
10. Davis TM. Diabetes and metabolic dysfunction-associated fatty liver disease. Metabolism 2021;123:154868.
47. Lin YC, Wu CC, Ni YH. New perspectives on genetic prediction
for pediatric metabolic associated fatty liver disease. Front Pediatr 2020;8:603654.
67. Tsou MT, Chen YJ. Gender-based association of coronary artery calcification and framingham risk score with non-alcoholic fatty liver disease and abdominal obesity in taiwanese adults, a cross-sectional study. Front Cardiovasc Med 2022;9:803967.
68. Hsiao CC, Teng PH, Wu YJ, Shen YW, Mar GY, Wu FZ. Severe, but not mild to moderate, non-alcoholic fatty liver disease associated with increased risk of subclinical coronary atherosclerosis. BMC Cardiovasc Disord 2022;21:244.


113. 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.
118. Chen YP, Lu FB, Hu YB, Xu LM, Zheng MH, Hu ED. A systematic review and a dose-response meta-analysis of coffee dose and...
135. Elsaid MI, Li Y, Bridges JFP, Brock G, Minacapelli CD, Rustgi VK. Association of bariatric surgery with cardiovascular outcomes in adults with severe obesity and nonalcoholic fatty liver disease. JAMA Netw Open 2022;5:e2235003.
141. Vuppalanchi R, Noureddin M, Alkhouri N, Sanyal AJ. Therapeutic pipeline in nonalcoholic steatohepatitis. Nat Rev Gastroen-
terol Hepatol 2021;18:373-392.


Comparison of four histological scoring systems for autoimmune hepatitis to improve diagnostic sensitivity

Soomin Ahn, Sook-Hyang Jeong, Eun Ju Cho, Kyoungbun Lee, Gilhyang Kim, and Haeryoung Kim

1Department of Pathology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam; 2Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 3Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam; Departments of Internal Medicine and Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul; 4Department of Pathology, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea

Graphical Abstract

CONCLUSION

- Although the 2008 International Autoimmune Hepatitis Group (IAIHG) simplified scoring system is commonly used in clinical practice, the histological component in this scoring system has low sensitivity for diagnosing autoimmune hepatitis.
- Substituting the histological component of the 2008 scoring system by modified histological scores (2017 UCSF or 2022 IAHPG) increased the diagnostic sensitivity, especially for AIH cases with acute hepatitis patterns.

Study Highlights

- Although the 2008 International Autoimmune Hepatitis Group (IAIHG) simplified scoring system is commonly used in clinical practice, the histological component in this scoring system has low sensitivity for diagnosing autoimmune hepatitis.
- Substituting the histological component of the 2008 scoring system by modified histological scores (2017 UCSF or 2022 IAHPG) increased the diagnostic sensitivity, especially for AIH cases with acute hepatitis patterns.
INTRODUCTION

The diagnosis of autoimmune hepatitis (AIH) is made based on the clinical presentation, laboratory findings (e.g., elevated liver enzymes, serum immunoglobulin G [IgG], and the presence of autoantibodies), the histopathological findings, and the exclusion of other etiologies. The diagnosis of AIH can at times be difficult considering the heterogeneity of the clinical features and the large number of differential diagnoses. Diagnostic scoring systems have been proposed by the International Autoimmune Hepatitis Group (IAIHG)—originally in 1993, revised in 1999 and then simplified in 2008—with the initial aim of selecting a homogeneous group of AIH patients for research. These scoring systems have eventually made their way into clinical practice, and the revised criteria (hereafter referred to as “1999 IAIHG”) and the simplified criteria (hereafter referred to as “2008 IAIHG”) are commonly used in routine clinical care.

Background/Aims: The histological criteria in the 1999 and 2008 scoring systems proposed by the International Autoimmune Hepatitis Group (IAIHG) have their inherent limitations in diagnosing autoimmune hepatitis (AIH). In this study, we evaluated the histology components of four scoring systems (1. revised original scoring system [“1999 IAIHG”], 2. simplified scoring system [“2008 IAIHG”], 3. modified histologic criteria [“2017 UCSF”], and 4. a new histologic criteria proposed by the International AIH Pathology Group [“2022 IAHPG”]) in AIH patients.

Methods: Medical records and liver biopsies were retrospectively reviewed for 68 patients from two independent medical institutions, diagnosed with AIH based on the 1999 IAIHG system between 2006 and 2016. The histological features were reviewed in detail, and the four histological scoring systems were compared.

Results: Out of the 68 patients, 56 (82.4%) patients met the “probable” or “definite” AIH criteria of the 2008 IAIHG system, and the proportion of histologic score 2 (maximum) was 40/68 (58.8%). By applying the 2017 UCSF criteria, the number of histology score 2 increased to 60/68 (88.2%), and “probable” or “definite” AIH cases increased to 61/68 (89.7%). Finally, applying the 2022 IAHPG histology score resulted in the highest number of cases with histologic score 2 (64/68; 94.1%) and with a diagnosis of “probable” or “definite” AIH (62/68; 91.2%).

Conclusions: The recently proposed UCSF/IAHPG histological criteria increased the histology score of AIH. Substituting the histology component of the 2008 IAIHG system with the 2022 IAHPG criteria increased the sensitivity for diagnosing AIH (≥“Probable AIH”) from 82.4% to 91.2%. (Clin Mol Hepatol 2024;30:37-48)

Keywords: Autoimmune hepatitis; Histology; Diagnosis

Corresponding author : Haeryoung Kim
Department of Pathology, Seoul National University College of Medicine, Seoul National University Hospital, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-740-8322, Fax: +82-2-765-5600; E-mail: haeryoung.kim@snu.ac.kr
https://orcid.org/0000-0002-4205-9081

Sook-Hyang Jeong
Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7029, Fax: +82-31-787-4052, E-mail: jsh@snubh.org
https://orcid.org/0000-0002-4916-7990

Editor: Atsumasa Komori, National Hospital Organization Nagasaki Medical Center, Japan
Received : Aug. 23, 2023 / Revised : Oct. 20, 2023 / Accepted : Nov. 10, 2023

Abbreviations:
AIH, autoimmune hepatitis; IgG, immunoglobulin G; IAIHG, International Autoimmune Hepatitis Group; IAHPG, International Autoimmune Hepatitis Pathology Group; ANA, antinuclear antibody; SMA, smooth muscle antibody; anti-LKM, anti-liver kidney microsome antibody type 1; AMA, anti-mitochondrial antibody

https://doi.org/10.3350/cmh.2023.0325
http://www.e-cmh.org
Liver biopsy is an essential part of the diagnosis of AIH, and this is reflected in the histology components of the 1999 IAIHG and 2008 IAIHG systems. For example, in the 2008 IAIHG system, in which a minimum of 6 points is necessary to call a case as at least “probable” AIH, liver histology is allocated up to 2 out of a total of 8 points. However, the histological criteria stated in this system has not been prospectively validated, and the diagnostic utility of hepatocyte rosette formation and emperipolesis has been questioned. To this end, modifications in the histological component of the 2008 IAIHG criteria have been recently proposed by pathologists. In 2017, Balitzer et al. proposed a modified histological criteria for AIH (hereafter referred to as “2017 UCSF”), based on the necroinflammatory activity and the degree of plasma cell infiltration, and excluding rosettes and emperipolesis. This modified histologic criteria increased the histology score for AIH cases, and applying the 2017 UCSF histologic criteria to the 2008 IAIHG system increased the diagnostic sensitivity of AIH. Recently, a group of liver pathologists (International Autoimmune Hepatitis Pathology Group) published a consensus statement for the histological diagnosis of AIH (hereafter referred to as “2022 IAHPG”).

In this study, we compared the four scoring systems—1999 IAIHG, 2008 IAIHG, 2008 IAIHG with 2017 UCSF histology criteria (2008 IAIHG+2017 UCSF), and 2008 IAIHG with 2022 IAHPG criteria (2008 IAIHG+2022 IAHPG)—in a retrospective cohort of AIH patients from two institutions in Korea.

**MATERIALS AND METHODS**

**Case selection**

Liver biopsy cases diagnosed between 2006 and 2016 at Seoul National University Hospital and Seoul National University Bundang Hospital were retrieved from the pathology database of each institution, by searching the keyword “autoimmune hepatitis” in pathology reports. Cases containing the terms “typical AIH”, “consistent with AIH”, “suggestive of AIH”, and “the possibility of AIH could be considered” were included in the search. The medical records for each patient were reviewed and the following information was recorded: age, sex, body mass index (BMI), viral hepatitis status, alcohol consumption history, medication history, symptoms at initial presentation, biochemical status at the time of biopsy, serum IgG level, the presence of auto-antibodies (antinuclear antibody [ANA], anti-smooth muscle antibody [SMA], anti-liver kidney microsome [LKM] type1 antibody, anti-mitochondrial antibody [AMA]), co-morbidities, and extrahepatic autoimmune disorders including Sjögren syndrome and autoimmune thyroiditis. Cases with other etiologies (e.g., alcoholic hepatitis, viral hepatitis, toxic hepatitis, primary biliary cholangitis, overlap syndrome and those with uncertain etiology were excluded. Liver biopsies with fewer than 6 portal tracts were excluded. The disease course and response to immunosuppressive therapy were also evaluated. Complete response was defined as the normalization of serum transaminase and immunoglobulin levels below the upper normal limit within 6 months of initial treatment. Finally, 68 patients with a final diagnosis of AIH based on the clinical features, laboratory findings and pathology were included in the study. All cases were interpreted as “definite” or “probable” AIH, based on the pretreatment aggregates scores of the 1999 IAIHG system. This study was approved by the Institutional Review Board of Seoul National University Hospital and Seoul National University Bundang Hospital (#H-2208-098-1350), and informed consent was waived due to the retrospective nature of the study.

**Histopathological evaluation**

Hematoxylin and eosin and Masson trichrome-stain slides of liver biopsies were reviewed by two liver pathologists (S.A and H.K). The necroinflammatory grade was determined for each case using the Ishak grading scheme as follows: interface activity (A0-A4), confluent necrosis (B1-B6), lobular necroinflammatory activity (C0-C4), and portal inflammation (D0-D4). The degree of fibrosis was recorded using the Batts-Ludwig scheme (scale 0-4). The presence of hepatocyte rosettes, emperipolesis, and the predominant inflammatory cells were evaluated. Rosettes were defined as hepatocytes arranged around a clearly identifiable luminal space. Emperipolesis was defined as the presence of lymphocytes or plasma cells within the cytoplasm of hepatocytes. A plasma cell cluster was defined as an aggregate of more than 5 plasma cells in one focus. The presence of bile duct injury, bile duct loss and steatosis were also evaluated. The histological features were reviewed independently, without knowledge of the other pathologist’s interpretation results, and after completing the first round of independent histo-
**Table 1. Histological criteria in the four scoring systems**

<table>
<thead>
<tr>
<th>1999 IAIHG</th>
<th>2008 IAIHG</th>
<th>2017 UCSF</th>
<th>2022 IAHPG</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Histology score (2) (“typical”)</strong></th>
<th><strong>Histology score (2)</strong></th>
<th>Likely AIH (2)</th>
<th>Likely AIH (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Interface hepatitis (moderate/severe) +3</td>
<td>Hepatitic picture with any of the following:</td>
<td>Portal lymphoplasmacytic infiltrate + one/both of:</td>
<td></td>
</tr>
<tr>
<td>- Predominantly lymphocytic infiltrate +1</td>
<td>1) Plasma cells (numerous/clusters)</td>
<td>1) &gt;mild interface hepatitis</td>
<td></td>
</tr>
<tr>
<td>- Hepatocyte rosettes +1</td>
<td>2) High necroinflammatory activity (interface activity ≥A3* and/or confluent necrosis ≥B2 and/or lobular activity ≥C3)</td>
<td>2) &gt;mild lobular inflammation</td>
<td></td>
</tr>
<tr>
<td>- None of the above -5</td>
<td></td>
<td>- in the absence of histological features suggestive of another liver disease</td>
<td></td>
</tr>
<tr>
<td>- Biliary changes -3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Other changes -3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Histology score (1) (“compatible”)</strong></th>
<th><strong>Histology score (1)</strong></th>
<th>Possible AIH (1)</th>
<th>Possible AIH (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picture of chronic hepatitis with lymphocytic infiltration without all 3 of the above features</td>
<td>1) Hepatitis with mild/moderate necroinflammatory activity with any of the following:</td>
<td>Portal lymphoplasmacytic infiltrate without either of the likely features 1 or 2 above,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Interface activity A2</td>
<td>- in the absence of histological features suggestive of another liver disease OR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Confluent necrosis B1</td>
<td>- with one/both of the likely features above</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) Lobular activity C2</td>
<td>- in the presence of histological features suggestive of another liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2) Copper and CK7 stains negative†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Histology score (0) (“atypical”)</strong></th>
<th><strong>Histology score (0)</strong></th>
<th>Unlikely AIH (0)</th>
<th>Unlikely AIH (0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Features suggestive of other diagnoses</td>
<td>Features not observed in AIH:</td>
<td>Portal hepatitis without either of the likely features above,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Florid bile duct lesions</td>
<td>- in the presence of histological features suggestive of another liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Bile duct loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Copper/CK7 positivity†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Modified from 3,4,7,8.

*Ishak grade. †Only for cases with Ishak fibrosis score <3, not applicable to acute cases.*
pathological slide review, discrepant cases were reviewed together at a multiheaded microscope.

**Application of AIH scoring systems**

For each case, the histology scores of the 1999 IAIHG system (maximum 5 points) and 2008 IAIHG system (maximum 2 points) were calculated. We then evaluated the modified histology scores of the 2017 UCSF and 2022 IAHPG proposals. The four scoring systems are summarized in Table 1. For the 2022 IAHPG system, we categorized “likely AIH” as 2 points, “possible AIH” as 1 point and “unlikely AIH” as 0 point, and applied these scores to the 2008 IAIHG system.

### RESULTS

**Patient characteristics**

The clinical features of the 68 patients are summarized in Table 2. All patients were Korean and 55 (80.9%) were female. The mean age at the time of diagnosis was 58 years (range: 20–87), and 16 (23.5%) patients had BMI >25 kg/m². Thirty-two (47.1%) patients presented with acute presentation or aggravation. Thirty-six (52.9%) patients were symptomatic at the time of diagnosis (including jaundice, nausea, and abdominal discomfort), and one (1.5%) patient displayed hepatic decompensation. Serum IgG levels were elevated in 53 (77.9%) patients, and ANA was detected in 65 (95.6%) cases. Three patients with ANA negativity showed increased serum IgG levels. Anti-SMA was detected in 20 (35.1%) out of 57 tested patients, and anti-LKM type 1 antibody was negative in all 33 tested patients. All patients were negative for AMA.

Sixteen patients also had extrahepatic autoimmune disease, including Sjögren’s syndrome (11.8%), systemic lupus erythematosus (7.4%), rheumatoid arthritis (1.5%), polymyositis (1.5%), and autoimmune thyroid disease (1.5%). All patients were treated with immunosuppressive therapy: the initial treatment was prednisolone for 52 (76.5%) patients, and prednisolone and azathioprine for 16 (23.5%) patients. Out of the 66 patients with available follow up information, 53 (80.3%) patients showed complete response to initial treatment, and 13 (19.7%) showed incomplete response for initial treatment. Glucocorticoid-induced side effects were identified in 20 (30.3%) patients. Co-morbidities included hypertension (35.3%), diabetes mellitus (13.2%), and osteoporosis (35.3%).

### Histopathological findings

The histopathological features of 68 patients are summarized in Table 3. Sixty-three (92.6%) cases showed at least
moderate portal activity (≥D2), and 60 (88.2%) cases demonstrated at least mild/moderate interface activity (≥A2) (Fig. 1A). Confluent necrosis (≥B2) was identified in 27 (39.7%) cases. Lobular necroinflammatory activity (≥C2) was observed in 65 (95.6%) cases. Fibrosis of any degree was present in 65 (95.6%) cases. Bridging fibrosis and cirrhosis was observed in 15 (22.1%) and 16 (23.5%) patients, respectively. The predominant inflammatory cell type was lymphoplasmacytic in 60 (88.2%) cases, and lymphocytic in 8 (11.8%) cases. None of the cases showed predominant neutrophilic or eosinophilic infiltration. At least one plasma cell was present in the portal tracts of all but one case, and plasma cell clusters in the portal tracts were identified in 51 (75.0%) patients. Lobular plasma cell infiltration was observed in 59 (86.8%) patients. Emperipolesis and hepatocellular rosettes were identified in 49 (72.1%) and 50 (73.5%) cases, respectively (Fig. 1B, C). Co-existing steatosis was identified in 18 (26.5%); 18 of these cases showed mild macrovesicular steatosis and one case demonstrated moderate macrovesicular steatosis. Perivenular and perisinusoidal fibrosis was identified in 3 (4.4%) and 2 (2.9%) cases, respectively. None of the cases demonstrated features of steatohepatitis. Bile duct damage of more than mild degree was identified in 3 (4.4%) cases; however, bile duct loss was not observed in any patient.

Comparison of four scoring systems

Based on the clinical and histological findings of each case, the AIH scores were calculated using the four different scoring methods. The results are summarized in Figure 2A, 2B and Table 4. For the 1999 IAIHG (revised original scoring system), the pretreatment criteria was applied. All patients met the definite or probable criteria by the 1999 IAIHG system (60.3% “definite”, 39.7% “probable”). The histologic score component ranged from 0 to 5 (the maximum histology score) with a mean value of 4.43. By this system, 50 (73.5%) cases were given the maximum histologic score (5).

Using the 2008 IAIHG system, 56 (82.4%) cases met the probable (total score ≥6) or definite (≥7) criteria for AIH (61.8% “definite”, 20.6% “probable”). The histological scores were “1” for 28 (41.2%) and “2” for 40 (58.8%) cases, and the mean score was 1.59.

By applying the 2017 UCSF system to the 2008 IAIHG system (2008 IAIHG+2017 UCSF), the number of cases that qualified for the maximum histology score (“2”) increased from 40 (58.8%) to 60 (88.2%) cases, and the mean histologic score increased from 1.59 to 1.87. Accordingly, when the 2017 UCSF histology criteria was applied to the histology component of the 2008 IAIHG system, the number of cases that met the definite or probable criteria for AIH increased to 61 (89.7%), with an increase of “definite” cases from 61.8% to 64.7%.

Finally, by the recently proposed 2022 IAHPG criteria (2008 IAIHG+2022 IAHPG), the number cases that qualified for the maximum histology score (“2”) was the highest at 64 (94.1%) cases, with the highest mean histologic score of 1.94. By applying the 2022 IAHPG criteria to the 2008 system, 62 (91.2%) cases met the probable or definite criteria for AIH, with 45 (66.2%) cases being classified as “definite AIH”. The proportion of definite AIH (total score ≥7) was the highest among four scoring systems.

For the thirty-two cases with acute presentation or aggravation, 21 (65.6%) cases met the probable or definite AIH category by 2008 IAIHG system (Table 5). However, by applying 2017 UCSF system, the number of cases that qualified for the maximum histology score (“2”) increased from 21 (65.6%) to 29 (90.6%) cases, and there was an increase in cases that met the probable or definite AIH category from 81.3% to 90.6%. Finally, by applying 2022 IAHPG system, the number of cases with the maximum histology score was the highest (96.9%), and the proportion of probable or definite AIH category was the highest (93.8%) (Fig. 2C, D).

DISCUSSION

Although the 1999 IAIHG scoring system is regarded as the gold standard for the diagnosis of AIH, it was originally intended for research purposes and is too complicated to use in everyday clinical practice. To this end, the Simplified criteria was proposed in 2008 to provide a diagnostic criteria that was easier to use; however, it has been recognized that the histology component of the 2008 IAIHG system may lead to underscoring of potential AIH cases, and proposals have been put forth to increase the diagnostic sensitivity of this system. In this retrospective study, we compared four AIH scoring systems in 68 Korean patients who were diagnosed as AIH based on the 1999 IAIHG system, and focused on the histological score component. As expected, the 2008 IAIHG system showed the lowest sensitivity (82.4% for “Probable or definite AIH”) among the four scoring systems.
### Table 3. Histopathological features (n=68)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Portal inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>D0 (minimal)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D1 (mild)</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>D2 (moderate)</td>
<td>26 (38.2)</td>
</tr>
<tr>
<td>D3 (moderate/marked)</td>
<td>29 (42.6)</td>
</tr>
<tr>
<td>D4 (marked)</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td><strong>Interface activity</strong></td>
<td></td>
</tr>
<tr>
<td>A0 (none)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>A1 (mild)</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>A2 (mild/moderate)</td>
<td>10 (14.7)</td>
</tr>
<tr>
<td>A3 (moderate)</td>
<td>29 (42.6)</td>
</tr>
<tr>
<td>A4 (severe)</td>
<td>21 (30.9)</td>
</tr>
<tr>
<td><strong>Confluent necrosis</strong></td>
<td></td>
</tr>
<tr>
<td>B0 (none)</td>
<td>33 (48.5)</td>
</tr>
<tr>
<td>B1 (focal confluent necrosis)</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>B2 (zone 3 necrosis in some areas)</td>
<td>4 (5.9)</td>
</tr>
<tr>
<td>B3 (zone 3 necrosis in most areas)</td>
<td>7 (10.3)</td>
</tr>
<tr>
<td>B4 (zone 3 necrosis+occasional portal–central bridging)</td>
<td>11 (16.2)</td>
</tr>
<tr>
<td>B5 (zone 3 necrosis+multiple portal–central bridging)</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>B6 (panacinar or multiacinar necrosis)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Spotty necrosis</strong></td>
<td></td>
</tr>
<tr>
<td>C0 (none)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C1 (one focus or less per ×10)</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>C2 (two to four foci per ×10)</td>
<td>20 (29.4)</td>
</tr>
<tr>
<td>C3 (five to ten foci per ×10)</td>
<td>28 (41.2)</td>
</tr>
<tr>
<td>C4 (more than 10 foci per ×10)</td>
<td>17 (25.0)</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td></td>
</tr>
<tr>
<td>No fibrosis</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Portal fibrosis</td>
<td>14 (20.6)</td>
</tr>
<tr>
<td>Periportal fibrosis</td>
<td>18 (26.5)</td>
</tr>
<tr>
<td>Septal fibrosis</td>
<td>15 (22.1)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>16 (23.5)</td>
</tr>
<tr>
<td>Co-existing perivenular fibrosis</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Co-existing perisinusoidal fibrosis</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td><strong>Not applicable</strong></td>
<td>2 (2.9)</td>
</tr>
<tr>
<td><strong>Predominant cell types</strong></td>
<td></td>
</tr>
<tr>
<td>Lymphoplasmacytes</td>
<td>60 (88.2)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
By substituting the histological criteria of the 2008 IAIHG system with the 2022 IAHPG histologic score, the sensitivity of diagnosing “definite AIH” and “at least probable AIH” increased from 61.8% and 82.4%, respectively (2008 IAIHG) to 66.2% and 91.2%, respectively (2008 IAIHG+2022 IAHPG). When the histological component was analyzed separately, the proportion of cases with the maximum histological scores was highest by the 2022 IAHPG method (94.1%), and lowest by the 2008 IAIHG system (58.8%).

Liver biopsy is an essential component in making a diagnosis of AIH, and each AIH scoring system accordingly contains a histology score. The 1999 IAIHG system emphasizes the importance of moderate/severe interface hepatitis (3 points out of a maximum of 5 histology points), and the total histology score is the result of a simple addition of component scores. Therefore, compared to the 2008 system in which potential AIH cases without hepatocytic rosettes or emperipoleisis are regarded insufficient for a diagnosis of “typical” AIH, such cases could qualify for a diagnosis of “definite AIH” by the 1999 system as long as other clinical or pathological features are present, yielding a total score of >15 points. In this regard, the 1999 IAIHG system is more sensitive compared to the 2008 IAIHG system. However, both IAIHG systems do not take into account the acute hepatitis presentation of AIH; indeed, the histological features of AIH vary according to disease status, and do not always demonstrate the classical feature of a chronic hepatitis pattern with portal lymphoplasmacytic infiltration and interface hepatitis. AIH with acute presentation mostly demonstrates histological features of acute lobular hepatitis, and such cases would be less likely to meet the histological criteria for AIH according to the 1999 and 2008 IAIHG systems, due to the lack of interface hepatitis. In fact, acute hepatitis patterns of AIH are being increasingly recognized, and the differential diagnosis between AIH and other causes of acute hepatitis, such as acute viral hepatitis and drug/toxin-induced liver injury, is often difficult but at the same time, very important. It is therefore crucial for pathologists to be aware of this type of AIH, and applying the 2022 IAHPG definitions for the lobular hepatitis pattern of AIH would serve as a useful guiding tool in identifying the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of plasma cell clusters</td>
<td>51 (75.0)</td>
</tr>
<tr>
<td>Presence of emperipolesis</td>
<td>48 (70.6)</td>
</tr>
<tr>
<td>Presence of hepatocyte rosettes</td>
<td>51 (75.0)</td>
</tr>
<tr>
<td>Presence of bile duct damage (more than mild degree)</td>
<td>3 (4.4)</td>
</tr>
<tr>
<td>Presence of steatosis</td>
<td>18 (26.5)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).
acute form of AlH.\textsuperscript{6}

For the 2008 IAIHG “simplified scoring” system, the histological score accounts for a maximum of 2 out of 8 points.\textsuperscript{4} A histological score of “2” requires the presence of interface hepatitis and hepatocytic rosettes and emperipolesis, and if any one of these features are absent, a score of 1 is given for a chronic hepatitis picture.\textsuperscript{4} However, recent studies have questioned the utility of including hepatocytic rosettes and emperipolesis as prerequisites for a diagnosis of “typical” AIH.\textsuperscript{7,18} The reported frequency of hepatocyte rosette formation and emperipolesis has markedly varied, with ranges of 19–75% and 19–80%, respectively.\textsuperscript{7,18,22} They are difficult to interpret in daily diagnostic practice: identifying emperipolesis, especially, is a time-consuming task for pathologists, and also prone to interobserver variability in interpretation. Moreover, hepatocytic rosettes and emperipolesis lack sensitivity and specificity for AIH, as hepatocyte rosettes are seen during regeneration after various types of injury, and em-

Figure 2. Validation of autoimmune hepatitis scoring systems. (A) Percentage of maximum histology score for total patients (n=68). By 1999 IAIHG criteria, 73.5% of cases were given the maximum histologic score (*maximum score: 5). The proportion of cases with the maximum histological scores were lowest by the 2008 IAIHG system (58.8%), and highest by the 2022 IAHPG method (94.1%). (B) Total score of four scoring systems for total patients (n=68). All patients met ≥”Probable AlH” criteria by the 1999 IAIHG system. In contrast, 82.4% of patients met ≥”Probable AlH” of the 2008 IAIHG system. However, substituting UCSF and IAHPG histologic criteria to the 2008 IAIHG system resulted in increased sensitivity for diagnosing AIH (≥”Probable AlH”) from 82.4% to 89.7% and 91.2%, respectively. (C) Percentage of maximum histology score for patients with acute presentation or aggravation, n=32. By the 1999 IAIHG criteria, 81.3% of cases were given the maximum histologic score (*maximum score: 5). The proportion of cases with the maximum histological scores were lowest by the 2008 IAIHG system (65.6%), and highest by applying the 2022 IAHPG method (96.9%). (D) Total score of four scoring systems for patients with acute presentation or aggravation, n=32. All patients met ≥”Probable AlH” criteria by the 1999 IAIHG system. In contrast, 81.3% of patients met ≥”Probable AlH” of the 2008 IAIHG system. However, substituting UCSF and IAHPG histologic criteria to the 2008 IAIHG system resulted in increased sensitivity for diagnosing AIH (≥”Probable AlH”) from 81.3% to 90.6% and 93.8%, respectively. IAIHG, international autoimmune hepatitis group; IAHPG, international autoimmune hepatitis pathology group; AlH, autoimmune hepatitis.
### Table 4. Validation of autoimmune hepatitis scoring systems for total patients (n=68)

<table>
<thead>
<tr>
<th></th>
<th>1999 IAIHG</th>
<th>2008 IAIHG</th>
<th>2008 IAIHG+2017 UCSF</th>
<th>2008 IAIHG+2022 IAHPG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7 (10.3)</td>
<td>11 (16.2)</td>
<td>50 (73.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>28 (41.2)</td>
<td>40 (58.8)</td>
<td>1 (1.5)</td>
<td>7 (10.3)</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>60 (88.2)</td>
<td>60 (88.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total score</td>
<td>Probable (≥10)</td>
<td>Definite (≥16)</td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
</tr>
<tr>
<td>68 (100)</td>
<td>41 (60.3)</td>
<td>56 (82.4)</td>
<td>42 (61.8)</td>
<td>61 (89.7)</td>
</tr>
<tr>
<td></td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
</tr>
<tr>
<td>62 (91.2)</td>
<td>45 (66.2)</td>
<td>64 (94.1)</td>
<td>44 (64.7)</td>
<td>62 (91.2)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).
IAIHG, International Autoimmune Hepatitis Group; IAHPG, International Autoimmune Hepatitis Pathology Group.

### Table 5. Validation of autoimmune hepatitis scoring systems for patients with acute presentation or aggravation (n=32)

<table>
<thead>
<tr>
<th></th>
<th>1999 IAIHG</th>
<th>2008 IAIHG</th>
<th>2008 IAIHG+2017 UCSF</th>
<th>2008 IAIHG+2022 IAHPG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (3.1)</td>
<td>5 (15.6)</td>
<td>26 (81.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>11 (34.4)</td>
<td>21 (65.6)</td>
<td>1 (3.1)</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>29 (90.6)</td>
<td>0 (0)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Total score</td>
<td>Probable (≥10)</td>
<td>Definite (≥16)</td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
</tr>
<tr>
<td>32 (100)</td>
<td>22 (68.8)</td>
<td>26 (81.3)</td>
<td>19 (59.4)</td>
<td>29 (90.6)</td>
</tr>
<tr>
<td></td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
<td>Probable (≥6)</td>
<td>Definite (≥7)</td>
</tr>
<tr>
<td>30 (93.8)</td>
<td>19 (59.4)</td>
<td>18 (56.3)</td>
<td>30 (93.8)</td>
<td>19 (59.4)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).
IAIHG, International Autoimmune Hepatitis Group; IAHPG, International Autoimmune Hepatitis Pathology Group.
peripolesis is seen in severe lobular injury. Thus, these two histological features were excluded from the 2017 UCSF and 2022 IAHPG histology scores.

The presence of hyaline globules in Kupffer cells has been suggested to serve as a diagnostic clue for AIH, and a modification of the 2008 IAIHG histology component has been proposed by Gurung et al., which requires the presence of Kupffer cell hyaline globules in addition to a prominent plasmacytic infiltration to qualify as a “typical” AIH. However, this needs further validation; a few other studies demonstrated that this feature was not correlated with serum IgG levels, autoantibody status or histological activity/fibrosis, and moreover, its identification requires additional stains such as periodic acid-Schiff post-diastase (D-PAS) stain and CD68. Another interesting finding which requires further validation is the higher number of apoptotic lymphocytes in portal tracts in AIH compared to other liver diseases.

The limitations of this study are as follows. It is a retrospective study from two institutions on a relatively small number of cases. In addition, a control group with other etiologies was not included, and only typical cases confirmed by the 1999 IAIHG system were included in this study. Therefore, the specificity of each diagnostic system was not assessed. Nevertheless, this is a uniform cohort of Korean AIH patients and, to our knowledge, this is the first study to directly compare the histological scoring systems, including the most recent 2022 IAHPG consensus criteria.

In summary, recently proposed 2017 UCSF and 2022 IAHPG criteria increased the histological scores of AIH cases, and substituting the histological component of the 2008 IAIHG system with these newly proposed histological criteria increased the diagnostic sensitivity for AIH. Therefore, the recently proposed histologic criteria are expected to resolve the low diagnostic sensitivity of 2008 simplified scoring system, which warrants further investigation.

Authors’ contribution

Acknowledgements
This study was supported by the research fund of Seoul National University Bundang Hospital (SNUBH 02-2018-011, S.A.) and The Research Supporting Program of The Korean Association for the Study of the Liver and The Korean Liver Foundation (H.K.).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Cardiovascular risk in chronic hepatitis B patients treated with tenofovir disoproxil fumarate or tenofovir alafenamide

Hyeyeon Hong, Won-Mook Choi, Danbi Lee, Ju Hyun Shim, Kang Mo Kim, Young-Suk Lim, Han Chu Lee, and Jonggi Choi

Department of Gastroenterology, Liver Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Study Highlights

- TAF and TDF affect the lipid profiles of patients with CHB differently.
- In the present study, there was no significant difference in the long-term risk of adverse cardiovascular outcomes between patients treated with TAF and those treated with TDF.
- Patients treated with TDF exhibited a significantly greater decline in median changes of TC, HDL, and triglyceride than those treated with TAF. However, the TC/HDL ratio did not show a significant difference between the TAF and TDF groups.
- Active smoking and a history of cardiovascular events were significantly associated with an increased risk of adverse cardiovascular outcomes.
- Despite the distinct serial changes in lipid profiles, long-term cardiovascular outcomes were comparable between the TAF and TDF treatments among patients with CHB.
INTRODUCTION

Potent oral antiviral agents for chronic hepatitis B (CHB) have been proven to prevent disease progression and decrease the development of hepatocellular carcinoma (HCC) via effective suppression of hepatitis B virus (HBV).¹,² Currently, entecavir, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) are widely used and recommended by international guidelines for the treatment of CHB.³⁻⁵ However, a functional cure of CHB, defined as hepatitis B surface antigen (HBsAg) seroclearance, regardless of the appearance of the hepatitis B surface antibody, rarely occurs with these oral antiviral agents. Therefore, long-term antiviral treatment is inevitable in patients with CHB. In addition, age of patients with CHB and their prevalence of comorbidities such as dyslipidemia, diabetes, hypertension, and chronic kidney disease have increased over the past decades.⁶ Indeed, metabolic risk factors are known to increase the risk of HCC in patients with CHB.⁷ Hence, increased attention is being paid to the monitoring and management of comorbidities in patients with CHB under long-term antiviral treatment.

TAF is the most recently approved antiviral agent for CHB. The phase 3 trial for TAF approval demonstrated that its efficacy was not inferior to TDF and that it showed a better safety profile in bone and kidney.⁸⁻¹⁰ However, in this trial, patients treated with TAF had comparable risks of cardiovascular outcomes, defined as MACE, as patients treated with TDF. (Clin Mol Hepatol 2024;30:49-63)

Keywords: Antiviral agent; Tenofovir; Lipid; Cardiovascular risk

Background/Aims: Tenofovir disoproxil fumarate (TDF) is known to have a lipid-lowering effect. This is in contrast to tenofovir alafenamide (TAF), which has a lipid-neutral effect. Therefore, concerns have been raised as to whether these differences affect long-term cardiovascular risk. Here, we aimed to evaluate the long-term risk of cardiovascular events in chronic hepatitis B (CHB) patients treated with TAF or TDF.

Methods: We retrospectively analyzed 4,124 treatment-naïve CHB patients treated with TDF (n=3,186) or TAF (n=938) between 2012 and 2022. The primary outcome was a composite endpoint of major adverse cardiovascular events (MACE), including myocardial infarction, ischemic stroke, and hospitalization for unstable angina or heart failure. Serial changes in lipid profiles between two treatments were also explored.

Results: The median age of the patients was 50.6 years, and 60.6% of the patients were male. At baseline, 486 (11.8%) and 637 (15.4%) of the patients had dyslipidemia and fatty liver, respectively. A total of 42 MACE occurred, with an annual incidence of 0.2%/100 person-years (PYS). At 1, 3, and 5 years, the cumulative risk of MACE was 0.4%, 0.8%, and 1.2% in patients treated with TDF, and 0.2%, 0.7%, and 0.7% in patients treated with TAF, respectively (P=0.538). No significant differences in the risk of MACE were observed between TDF and TAF. A multivariable analysis found that current smoker and a history of cardiovascular events were risk factors associated with an increased risk of MACE.

Conclusions: Patients treated with TAF had comparable risks of cardiovascular outcomes, defined as MACE, as patients treated with TDF. (Clin Mol Hepatol 2024;30:49-63)

Keywords: Antiviral agent; Tenofovir; Lipid; Cardiovascular risk

Abbreviations:
AHR, adjusted hazard ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAD, coronary artery disease; CHB, chronic hepatitis B; eGFR, estimated glomerular filtration rate; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; HF, heart failure; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; MACE, major adverse cardiovascular event; MI, myocardial infarction; PS, propensity score; TAF, tenofovir alafenamide; TC, total cholesterol; TDF, tenofovir disoproxil fumarate; TG, triglyceride

Corresponding author : Jonggi Choi
Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-1328, Fax: +82-2-485-5782, E-mail: j.choi@amc.seoul.kr
https://orcid.org/0000-0002-7470-5850

Editor: Ming-Lung Yu, Kaohsiung Medical University Hospital, Taiwan
Received : Aug. 25, 2023 / Revised : Nov. 19, 2023 / Accepted : Nov. 19, 2023

including levels of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG). In contrast, no significant differences were observed in the lipid profiles of patients treated with TAF. These patterns of changes in lipid profiles were consistently reproduced in real-world data from Korean patients. In general, previous studies, which are mostly from patients with human immunodeficiency virus (HIV), have shown that TDF exerts a consistent lipid-lowering effect. In contrast, TAF is known to have a minimal effect on lipid profiles. Nevertheless, concerns have recently been raised about whether these changes in lipid profiles affect the risk of long-term cardiovascular events in patients with CHB, since to date, this has not been well-studied.

In this study, we aimed to evaluate the risk of long-term cardiovascular events between TAF and TDF treatments and identify factors associated with cardiovascular events in patients with CHB in a large real-world cohort.

MATERIALS AND METHODS

Study design and study population

This study was a historical cohort study using data from adult treatment-naïve CHB patients who were either treated with TAF (25 mg/day) or TDF (300 mg/day) at Asan Medical Center, Seoul, Republic of Korea between January 2012 and May 2022. Patients meeting the following criteria were included in this study: (i) aged 18 years or older; (ii) HBsAg-positive for more than 6 months; (iii) no prior use of an oral antiviral agent for CHB; and (iv) treatment duration more than 6 months. Patients meeting any of the following criteria were excluded: (i) coinfection with hepatitis C virus, hepatitis D virus, HIV, or other hepatotropic viruses; and (ii) liver transplantation or solid organ transplantation. After screening patient records for inclusion and exclusion criteria, a total of 4,124 patients was included in the present study.

This study was approved by the institutional review board of Asan Medical Center (IRB Approval Number: 2022-0463) and was exempted from obtaining consent because of the retrospective nature of the patient evaluations. Moreover, we followed the guidelines for the reporting of observational studies in epidemiology (Supplementary Table 1).

Clinical and laboratory variables

Demographic variables characterizing the study population included age, sex, height, weight, body mass index, and smoking status. Comorbidities at baseline, including diabetes mellitus, hypertension, dyslipidemia, and a history of myocardial infarction (MI), stroke, heart failure (HF), and coronary artery disease (CAD) were manually reviewed using the electronic medical record database at Asan Medical Center. Dyslipidemia was defined as a history of dyslipidemia in the medical record, a total cholesterol level of 240 mg/dL or higher, or using a lipid-lowering agent. Information regarding medications, including aspirin, clopidogrel, and lipid-lowering agents, were also obtained. Laboratory data included platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, prothrombin time, TC, TG, HDL, LDL, creatinine, and estimated glomerular filtration rate (eGFR) as determined by the CKD-EPI equation. HBV-related variables included hepatitis B e antigen (HBeAg) and serum HBV DNA. Serum HBV DNA levels were measured using real-time PCR (linear dynamic detection range, 15-1x10^9 IU/mL; Abbott Laboratories, Chicago, IL, USA). Cirrhosis was clinically defined as the presence of any cirrhotic features, including coarse liver echotexture and nodular liver surface on ultrasonography, clinical features of portal hypertension (e.g., ascites, splenomegaly, or varices), or thrombocytopenia (<150,000/mm^3). A fatty liver was determined by the detection of hyper-echogenicity by ultrasonography.

Study outcome and follow-up strategy

The primary outcome was a composite of major adverse cardiovascular events (MACE), which included MI, ischemic stroke, and hospitalization for unstable angina or HF. The index date of this study was the date of oral antiviral agent initiation. Patients received regular follow-up sessions, including a routine clinical examination, liver function tests, and imaging tests for HCC surveillance at least every 3–6 months. Patients were followed up until the development of MACE, death from any cause, liver transplantation, the last scheduled follow-up date, or November 15, 2022, whichever came first.
**Statistical analysis**

Baseline characteristics were summarized using frequencies and percentages for categorical variables and means and standard deviations for continuous variables. When characterizing baseline characteristics between the two treatments, t-tests or Mann–Whitney U-tests were used to evaluate the statistical significance of differences in continuous variables, and chi-square or Fisher’s exact tests were used to assess the statistical significance of differences in categorical variables. Cumulative incidences of MACE between the two treatments were estimated using the Kaplan–Meier method and were compared using a log-rank test. In addition, we used Cox proportional hazard models to identify risk factors for MACE development. Propensity score (PS) matching was used to minimize confounding variables between the two treatments. Multiple imputation was used to estimate missing values; these values comprised 4.83–7.45% of the baseline laboratory data. PS was computed using the following 21 variables: age; sex; diabetes; hypertension; history of stroke; history of MI; history of HF; history of CAD; cirrhosis; fatty liver; TC; platelet count; AST; ALT; total bilirubin; albumin; creatinine; HBeAg positivity; aspirin use; clopidogrel use; lipid-lowering agent use. Finally, nearest-neighbor 1:1 matching was performed with a caliper size of 0.1.

In addition, we also performed two subgroup analyses for sensitivity analysis. First, patients who did not receive any lipid-lowering agents at baseline or during the follow-up period were analyzed to determine whether there were serial changes in their lipid profiles. Second, we compared the observed changes in lipid profiles among our study population to untreated patients with CHB as a control. A total of 4,309 untreated patients with CHB was used for this sensitivity analysis, and their baseline characteristics are presented in Supplementary Table 2. For all statistical analyses, P-values <0.05 were considered statistically significant, and all statistical analyses were conducted using R version 4.3.0 (https://www.r-project.org).

**RESULTS**

**Baseline characteristics of the study population and propensity-score matched cohort**

Our study population comprised 4,124 treatment-naïve patients with CHB. The baseline characteristics of these patients are presented in Table 1. The median follow-up period was 4.1 years. The median patient age was 50.6 years, and 60.6% of patients were male. At baseline, 382 (9.3%), 613 (14.9%), and 60 (1.5%) patients had diabetes, hypertension, and CAD, respectively. Among the 3,269 patients for whom smoking history data was available, 745 (18.1%), 613 (14.9%), and 1,911 (46.3%) patients were current, former, and never smokers, respectively.

TAF was initiated in 938 patients, and 3,186 patients were initially treated with TDF. Compared with the TDF treatment group, the TAF treatment group had a smaller proportion of patients who were male, had cirrhosis and HCC. However, patients who received TAF had a higher prevalence of dyslipidemia and fatty liver, and the administration of lipid-lowering agents than those that received TDF treatment. The TAF treatment group had significantly higher levels of TC, HDL, LDL, and TG than the TDF treatment group. Finally, PS-matching generated 911 pairs to enable comparisons between the two treatments. We did not observe statistically significant differences in the baseline characteristics of each treatment group, as shown in Table 1.

**MACE**

During the 15,527 person-years (PYs) of observation, 42 MACE occurred with an annual incidence of 0.27/100 PYs. The cumulative incidence of MACE in the entire study population was 0.4%, 0.8%, 1.2%, and 1.7% at 1, 3, 5, and 7 years, respectively.

Of the 42 occurrences of MACE, unstable angina requiring coronary artery evaluation with hospitalization was the most frequent (n=24), followed by ischemic stroke (n=14), MI (n=3), and HF requiring admission (n=1). Patients who developed MACE were significantly older and had a higher prevalence of diabetes, hypertension, and a history of CAD compared to patients who were not afflicted by a MACE (Supplementary Table 3). Critically, no significant differences in the rate of
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Entire population</th>
<th>Propensity score-matched cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=4,124)</td>
<td>TAF (n=938)</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50.6 (42.1–57.8)</td>
<td>50.3 (42.0–58.4)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>2,501 (60.6)</td>
<td>505 (53.8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.5 (159.7–172.6)</td>
<td>166.5 (159.1–172.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.0 (57.6–74.5)</td>
<td>66.4 (57.0–75.5)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9 (21.9–26.1)</td>
<td>24.0 (22.0–26.3)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>122.0 (109.0–134.0)</td>
<td>126.0 (114.0–138.0)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73.0 (65.5–81.0)</td>
<td>75.5 (69.0–83.0)</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>1,911 (46.3)</td>
<td>481 (51.3)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>745 (18.1)</td>
<td>160 (17.1)</td>
</tr>
<tr>
<td>Past smoker</td>
<td>613 (14.9)</td>
<td>108 (11.5)</td>
</tr>
<tr>
<td>Not available</td>
<td>855 (20.7)</td>
<td>189 (20.1)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>382 (9.3)</td>
<td>84 (9.0)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>613 (14.9)</td>
<td>131 (14.0)</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>486 (11.8)</td>
<td>181 (19.3)</td>
</tr>
<tr>
<td>Stroke, n (%)</td>
<td>24 (0.6)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Myocardial infarction, n (%)</td>
<td>9 (0.2)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>19 (0.5)</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>60 (1.5)</td>
<td>13 (1.4)</td>
</tr>
<tr>
<td>Cirrhosis, n (%)</td>
<td>1,675 (40.6)</td>
<td>329 (35.1)</td>
</tr>
<tr>
<td>Fatty liver, n (%)</td>
<td>637 (15.4)</td>
<td>215 (22.9)</td>
</tr>
<tr>
<td>HCC, n (%)</td>
<td>281 (6.8)</td>
<td>15 (1.6)</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, ×10⁹/µL</td>
<td>167.0 (120.0–211.0)</td>
<td>178.0 (142.5–218.0)</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>56.0 (35.0–93.0)</td>
<td>52.5 (34.0–88.0)</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>57.0 (30.5–122.0)</td>
<td>62.5 (32.0–124.0)</td>
</tr>
<tr>
<td>Prothrombin time, INR</td>
<td>1.1 (1.0–1.2)</td>
<td>1.0 (1.0–1.1)</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Entire population</td>
<td>Propensity score-matched cohort</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td></td>
<td>Total (n=4,124)</td>
<td>TAF (n=938)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.9 (3.5–4.1)</td>
<td>3.9 (3.7–4.1)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.8 (0.6–1.1)</td>
<td>0.7 (0.6–0.9)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8 (0.7–0.9)</td>
<td>0.8 (0.7–0.9)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>101.0 (93.0–109.0)</td>
<td>100.0 (91.0–108.0)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>169.0 (144.0–194.0)</td>
<td>183.0 (160.0–207.0)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>51.0 (41.0–61.0)</td>
<td>55.0 (45.0–65.0)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>108.0 (84.0–132.0)</td>
<td>115.0 (98.0–136.0)</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>87.0 (68.0–117.0)</td>
<td>90.0 (72.0–119.5)</td>
</tr>
<tr>
<td>HBeAg-positive, n (%)</td>
<td>1,742 (47.4)</td>
<td>407 (45.4)</td>
</tr>
<tr>
<td>HBV DNA, log₁₀U/mL</td>
<td>5.5 (3.3–7.1)</td>
<td>5.6 (3.9–7.3)</td>
</tr>
</tbody>
</table>

Table 1. Continued

Aspirin, n (%)            | 108 (2.6)          | 25 (2.7)                      | 83 (2.6) | 0.990 |
| Clopidogrel, n (%)       | 53 (1.3)           | 11 (1.2)                      | 42 (1.3) | 0.855 |
| Lipid-lowering agent, n (%)| 329 (8.0)      | 107 (11.4)                    | 222 (7.0) | <0.001 |

Data are presented as a frequency and proportion or as a mean value with interquartile range. ASD, absolute standardized difference; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; BP, blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.
MACE occurrence were observed between the TAF and TDF treatments ($P=0.134$, Supplementary Table 3).

In the TAF group, MACE developed in five patients, in contrast, 37 patients experienced MACE in the TDF group. At 1, 3, and 5 years, the cumulative incidence of MACE was 0.4%, 0.8%, and 1.2% in patients treated with TDF, and 0.2%, 0.7%, and 0.7% in patients treated with TAF, respectively. No statistically significant differences in the risk of MACE were found when comparing the TAF and TDF treatments ($P=0.538$) (Fig. 1A).

Among the 911 PS-matched pairs, five patients in the TAF group and seven patients in the TDF group experienced MACE during the observation period. The cumulative risk of MACE in each group did not significantly differ in the PS-matched pairs ($P=0.820$, Fig. 1B).

**Risk factors for MACE development**

According to the univariate Cox model analysis, older age (hazard ratio [HR]: 1.07, 95% confidence interval [CI]: 1.04–1.10, $P<0.001$), diabetes, hypertension, and a history of cardiovascular events were all significantly associated with an increased risk of MACE (Table 2, $P<0.001$ for all). In the multivariate Cox model, significant factors for an increased risk of MACE were current smoker (adjusted hazard ratio [AHR]: 2.25, 95% CI: 1.07–4.75, $P=0.033$), hypertension (AHR: 2.07, 95% CI: 1.03–4.13, $P=0.040$), and a history of cardiovascular events (AHR: 29.2, 95% CI: 14.7–57.9, $P<0.001$, Table 2). Diabetes appeared to be associated with a higher risk of MACE despite not reaching statistical significance. When comparing TDF to the reference TAF treatment, we found no significant association with an increased risk of MACE in univariate analysis.

**Changes in lipid profiles**

In the entire study population, the median changes in TC from the baseline after 1, 2, 3, and 4 years of treatment were $-6$ mg/dL, $-9$ mg/dL, $-8$ mg/dL, and $-8$ mg/dL in the TDF group and 0 mg/dL, 0 mg/dL, 3 mg/dL, and 0 mg/dL in the TAF group, respectively (Table 3 and Supplementary Fig. 1A). In the PS-matched pairs, the median changes in TC from the baseline were $-12$ mg/dL, $-15$ mg/dL, $-13$ mg/dL, and $-15$ mg/dL in the TDF group and 3 mg/dL, 2 mg/dL, 4 mg/dL, and 0 mg/dL in the TAF group at 1, 2, 3, and 4 years of treatment, being statistically significant difference between the two treatments (Table 3 and Fig. 2A).

The median decrease in HDL levels from the baseline during treatment was significantly greater for the TDF treatment than the TAF treatment in both the entire population ($P<0.05$ for all, Table 3 and Supplementary Fig. 1B) and in the PS-matched pairs ($P<0.001$ for all, Table 3 and Fig. 2B).

Moreover, we found that LDL levels did not significantly differ between the two treatments in the entire population (Supplementary Fig. 1C), while the TDF treatment appeared to show a greater median decrease from the baseline than the TAF treatment in the PS-matched pairs (Table 3 and Fig. 2C).

Compared to the TAF treatment, the TDF treatment exhibited a significantly greater decline in median TG change from the baseline, both in the entire population (Supplementary Fig. 1D) and in the PS-matched pairs (Table 3 and Fig. 2D).

No significant difference was observed in the ratio of TC/HDL between the two treatments, either in the entire population (Supplementary Fig. 1E) or the PS-matched pairs (Table 3 and Fig. 2E).

**Subgroup analysis**

Of the 4,124 patients, 329 (8.0%) patients received lipid-lowering agents at baseline, and 296 (7.2%) patients began any types of lipid-lowering agent treatment during the follow-up period. Therefore, 3,499 (85.9%) patients who never administered lipid-lowering agents were included in the first subgroup analysis (Supplementary Table 4). In this subgroup, the TDF treatment showed a significantly greater decrease in median TC changes relative to TAF treatment during the study period (Supplementary Table 5 and Supplementary Fig. 2A). We also observed a significantly greater decrease in the median HDL, LDL, and TG levels during TDF treatment than in TAF treatment (Supplementary Fig. 2B–D). However, no significant difference was observed in the serial change in the TC/HDL ratio between the two treatments ($P>0.05$ for all, Supplementary Fig. 2E).

We compared these study population to 4,309 untreated CHB patients in our center as a sensitivity analysis. As shown in Figure 2F, the TDF treatment showed a greater decrease in median TC relative to untreated patients ($P<0.001$ for all). However, the TAF treatment did not significantly affect the median change in TC levels relative to untreated patients during the follow-up period ($P>0.05$ for all).
Figure 1. Cumulative probability of long-term cardiovascular outcomes in patients treated with tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF). (A) Between TAF and TDF treatment in the entire study population. (B) Between TAF and TDF treatment in the propensity-score matched pairs. MACE, major adverse cardiovascular event.
In this study, an analysis of large-scale, real-world data of treatment-naïve CHB patients treated with TAF or TDF revealed no significant difference in the long-term risk of adverse cardiovascular outcomes, defined as MACE, between the two treatments. Compared with TAF treatment, the TDF treatment resulted in a significantly greater decrease in median TC, HDL, and TG, and tended to also show a greater decrease in the median change of LDL. However, the TC/HDL ratio did not significantly differ between the two treatments. Active smoker, history of MI, and CAD—all of which are regarded as traditional risk factors for MACE—were found to be significantly associated with an increased risk of MACE.

Table 2. Factors associated with the risk of cardiovascular events

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Antiviral treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAF</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>TDF</td>
<td>1.35 (0.52–3.52)</td>
<td>0.500</td>
</tr>
<tr>
<td>Age, per 1 year increase</td>
<td>1.07 (1.04–1.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.61 (0.82–3.14)</td>
<td>0.200</td>
</tr>
<tr>
<td>Fatty liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1.24 (0.54–2.83)</td>
<td>0.600</td>
</tr>
<tr>
<td>Not available</td>
<td>1.57 (0.62–3.95)</td>
<td>0.300</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.80 (0.87–3.70)</td>
<td>0.110</td>
</tr>
<tr>
<td>Past smoker</td>
<td>1.41 (0.62–3.23)</td>
<td>0.400</td>
</tr>
<tr>
<td>Not available</td>
<td>0.22 (0.07–0.75)</td>
<td>0.015</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.99 (0.99–1.00)</td>
<td>0.087</td>
</tr>
<tr>
<td>HDL</td>
<td>0.97 (0.95–0.99)</td>
<td>0.010</td>
</tr>
<tr>
<td>LDL</td>
<td>0.99 (0.98–1.00)</td>
<td>0.074</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.00 (1.00–1.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>BMI&lt;25 kg/m²</td>
<td>0.94 (0.46–1.95)</td>
<td>0.900</td>
</tr>
<tr>
<td>BM≥25 kg/m²</td>
<td>1.58 (0.75–3.31)</td>
<td>0.200</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4.87 (2.53–9.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6.42 (3.49–11.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of cardiovascular event</td>
<td>54.5 (29.5–101.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; AHR, adjusted hazard ratio; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index.
### Table 3: Changes in lipid profiles during the study period

<table>
<thead>
<tr>
<th>Lipid profiles</th>
<th>TAF 25 mg</th>
<th>TDF 300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Median (IQR)</td>
<td>N Median (IQR)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change at month 6</td>
<td>904 1 (–13 to 18)</td>
<td>2,984 3 (–31 to 7)</td>
</tr>
<tr>
<td>Change at month 12</td>
<td>755 0 (–19 to 17)</td>
<td>2,535 2 (–25 to 13)</td>
</tr>
<tr>
<td>Change at month 18</td>
<td>663 2 (–21 to 18)</td>
<td>2,391 2 (–27 to 13)</td>
</tr>
<tr>
<td>Change at month 24</td>
<td>554 2 (–19 to 23)</td>
<td>2,309 2 (–27 to 14)</td>
</tr>
<tr>
<td>Change at month 30</td>
<td>461 1 (–18 to 24)</td>
<td>2,109 1 (–27 to 14)</td>
</tr>
<tr>
<td>Change at month 36</td>
<td>382 3 (–18 to 21)</td>
<td>1,979 0 (–27 to 13)</td>
</tr>
<tr>
<td>Change at month 42</td>
<td>313 0 (–21 to 18)</td>
<td>1,877 0 (–29 to 15)</td>
</tr>
<tr>
<td>Change at month 48</td>
<td>207 0 (–23 to 10)</td>
<td>1,607 0 (–28 to 15)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change at month 6</td>
<td>357 0 (–13 to 18)</td>
<td>1,296 2 (–11 to 1)</td>
</tr>
<tr>
<td>Change at month 12</td>
<td>382 0 (–13 to 18)</td>
<td>995 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 18</td>
<td>318 1 (–13 to 18)</td>
<td>887 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 24</td>
<td>252 0 (–13 to 18)</td>
<td>804 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 30</td>
<td>215 0 (–13 to 18)</td>
<td>704 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 36</td>
<td>167 0 (–12 to 18)</td>
<td>680 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 42</td>
<td>140 0 (–12 to 18)</td>
<td>627 0 (–5 to 1)</td>
</tr>
<tr>
<td>Change at month 48</td>
<td>81 0 (–10 to 0)</td>
<td>596 0 (–5 to 0)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change at month 6</td>
<td>486 0 (–6 to 10)</td>
<td>1,350 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 12</td>
<td>333 0 (–6 to 10)</td>
<td>971 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 18</td>
<td>280 0 (–6 to 10)</td>
<td>907 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 24</td>
<td>231 0 (–6 to 10)</td>
<td>828 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 30</td>
<td>216 0 (–6 to 10)</td>
<td>798 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 36</td>
<td>186 0 (–6 to 10)</td>
<td>758 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 42</td>
<td>154 0 (–6 to 10)</td>
<td>717 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 48</td>
<td>123 0 (–6 to 10)</td>
<td>677 0 (–6 to 10)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change at month 6</td>
<td>486 0 (–6 to 10)</td>
<td>1,350 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 12</td>
<td>333 0 (–6 to 10)</td>
<td>971 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 18</td>
<td>280 0 (–6 to 10)</td>
<td>907 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 24</td>
<td>231 0 (–6 to 10)</td>
<td>828 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 30</td>
<td>216 0 (–6 to 10)</td>
<td>798 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 36</td>
<td>186 0 (–6 to 10)</td>
<td>758 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 42</td>
<td>154 0 (–6 to 10)</td>
<td>717 0 (–6 to 10)</td>
</tr>
<tr>
<td>Change at month 48</td>
<td>123 0 (–6 to 10)</td>
<td>677 0 (–6 to 10)</td>
</tr>
</tbody>
</table>
Table 2. Continued

<table>
<thead>
<tr>
<th>Lipid profiles</th>
<th>Entire population</th>
<th>Propensity score matched cohort</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAF 25 mg</td>
<td>TDF 300 mg</td>
<td>P-value</td>
<td>TAF 25 mg</td>
<td>TDF 300 mg</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Median (IQR)</td>
<td>N</td>
<td>Median (IQR)</td>
<td>N</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>Change* at month 6</td>
<td>540</td>
<td>0.02 (-0.18 to 0.45)</td>
<td>1,341</td>
<td>0.04 (-0.28 to 0.48)</td>
<td>0.615</td>
<td>416</td>
</tr>
<tr>
<td>Change* at month 12</td>
<td>378</td>
<td>0.17 (-0.21 to 0.52)</td>
<td>955</td>
<td>0.14 (-0.38 to 0.58)</td>
<td>0.332</td>
<td>356</td>
</tr>
<tr>
<td>Change* at month 18</td>
<td>312</td>
<td>0.17 (-0.31 to 0.56)</td>
<td>840</td>
<td>0.17 (-0.33 to 0.59)</td>
<td>0.982</td>
<td>323</td>
</tr>
<tr>
<td>Change* at month 24</td>
<td>252</td>
<td>0.12 (-0.26 to 0.57)</td>
<td>800</td>
<td>0.14 (-0.33 to 0.62)</td>
<td>0.822</td>
<td>263</td>
</tr>
<tr>
<td>Change* at month 30</td>
<td>209</td>
<td>0.11 (-0.37 to 0.56)</td>
<td>704</td>
<td>0.19 (-0.31 to 0.67)</td>
<td>0.178</td>
<td>227</td>
</tr>
<tr>
<td>Change* at month 36</td>
<td>165</td>
<td>0.09 (-0.32 to 0.54)</td>
<td>675</td>
<td>0.17 (-0.35 to 0.65)</td>
<td>0.316</td>
<td>190</td>
</tr>
<tr>
<td>Change* at month 42</td>
<td>133</td>
<td>0.05 (-0.45 to 0.47)</td>
<td>625</td>
<td>0.24 (-0.31 to 0.68)</td>
<td>0.045</td>
<td>162</td>
</tr>
<tr>
<td>Change* at month 48</td>
<td>76</td>
<td>0.01 (-0.62 to 0.45)</td>
<td>590</td>
<td>0.24 (-0.30 to 0.74)</td>
<td>0.045</td>
<td>93</td>
</tr>
</tbody>
</table>

TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; IQR, interquartile range; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*Indicates change from the baseline.
Figure 2. Changes in the lipid profiles. (A) Total cholesterol in propensity-score (PS) matched pairs. (B) High-density lipoprotein in PS-matched pairs. (C) Low-density lipoprotein in PS-matched pairs. (D) Triglyceride in PS-matched pairs. (E) Total cholesterol/high-density lipoprotein ratio in PS-matched pairs. (F) Total cholesterol in patients with and without antiviral treatment. TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

due to the loss of the suppressive effect of TDF on lipid parameters—without displaying a significant change in their TC/HDL ratio. Nevertheless, apprehension regarding increasing the risk of cardiovascular events is often raised due to
long-term use of TAF accompanied by worsening lipid profiles, which is strongly associated with atherosclerosis. Interestingly, this concern originated among people living with HIV (PLWH) who require long-term antiviral treatment, as do patients with CHB.

Traditionally, a single lipid parameter, such as the LDL level, had been used to predict cardiovascular risk. However, according to a recent large epidemiologic study, the TC/HDL ratio is considered to be a better cardiovascular event predictor than the LDL level. In particular, adding LDL to the TC/HDL ratio does not increase its predictive power with respect to cardiovascular risk prediction. As shown in the phase 3 study for TAF approval in patients with CHB, no significant differences were observed in the TC/HDL ratios of patients who had up to three years of treatment. In the present study, we also observed that the TC/HDL ratio did not significantly differ between the two treatments in a patient population with a median follow-up duration of four years. This translates into a comparable cumulative risk of MACE, regardless of LDL levels, in TAF and TDF treatment groups.

Many predictive scores for predicting long-term cardiovascular risk have been developed and used for selecting patients for treatment, targeting treatment goals, and for prognosis. For example, the atherosclerotic cardiovascular disease (ASCVD) risk score is widely used. A previous study comparing TAF and TDF in treatment-naive PLWH showed that lipid changes associated with TDF treatment did not substantively affect cardiovascular risk profiles compared with TAF treatment. In addition, the findings of that study suggested that cardiovascular risk was not related to lipid changes between the two treatments but rather to the presence of traditional cardiovascular risk factors such as smoking or hypertension. Indeed, being a current smoker, having a history of MI or CAD, and having hypertension were all associated with an increased risk of cardiovascular events regardless of the type of antiviral treatment administered in the present study, despite hypertension showing marginal statistical significance between treatments.

Although TAF and TDF are both tenofovir prodrugs, questions regarding the differences in their effects on patient lipid profiles remain unanswered. An in vitro study demonstrated that TDF, compared with entecavir treatment and control, reduced supernatant cholesterol, activated PPAR-a-mediated signaling, and upregulated the expression of PPAR-a-target genes, including carnitine palmitoyltransferase 1 (CPT1) and CD36. This study also suggested that silencing of hepatic CD36 and PPAR-a signaling negated the lipid-lowering effect of TDF. However, the mechanism by which TAF affects lipid profiles has not been postulated so far, and whether the hypothesis of the above-mentioned study is also applicable to TAF requires further investigation.

Due to the increasing age of patients with CHB and the necessity of long-term antiviral treatment for most CHB patients, the management of comorbidities, including hypertension, diabetes, and hyperlipidemia, becomes more crucial. Cardiovascular events emerge as a major cause of death in virologically suppressed CHB patients without the development of HCC. Therefore, a thorough checkup, including an investigation of patient metabolic components (i.e., such as lipid profiles), may be added to routine care for patients with CHB regardless of the type of antiviral treatment used. Moreover, concerns regarding increased lipid levels resulting from TAF treatment may be diminished based on our findings, which suggests that there is no significant difference in lipid levels between TAF and no antiviral treatment.

The strengths of our study include the use of a large sample, which enabled us to obtain a sufficient number of primary outcomes despite the very low incidence of MACE. Given that TAF has recently been approved for CHB treatment, most previous studies reported data for lipid profiles as surrogate markers of long-term cardiovascular events. However, here, we investigated the association between two antiviral treatments and hard outcomes of CHB patients, which should ultimately be explored in greater detail in the future. In addition, we conducted a sensitivity analysis of patients who never received lipid-lowering agents to avoid the potential effect of lipid-lowering agents. Finally, our study population also compared treated and untreated CHB patients to explore the precise effect of TAF on patient lipid profiles.

However, the current study also has some limitations. First, as a retrospective single-center study based on observational data, there were possible selection biases. Also, not all patients were regularly followed-up with full lipid profiles unless some reasonable circumstance suggested that they be performed. However, the TC levels of almost all patients were available for analysis, and they showed trends that were consistent with those of previous studies. Second, factors that may interfere with lipid profiles, including alcohol consumption and weight changes, were not investigated in this study. Third, we could not assess the longitudinal trend of
the ASCVD score, which has been widely used for predicting the 10-year risk of cardiovascular events, because of the lack of variables that are components of the ASCVD score in some patients due to the nature of retrospective study. Fourth, our study population may not be adequately addressing the risk of cardiovascular outcomes considering the relatively young age of the study population (a median age of 50 years) because cardiovascular events occur more frequently with increasing age. However, previous studies consisting of treatment-naive patients with CHB generally included patients aged 40–50 years.8,9 Therefore, to evaluate and compare such outcomes in a prospective manner is not feasible. That is why we designed our study with a large number of patients and a relatively long period of follow-up. We believe that in order to strengthen and validate our findings, a population-level cohort study should be warranted in the future. Last, although we gathered information regarding all prescribed drugs from patient medical records, data related to lipid-lowering agents that may have been prescribed by other hospitals or obtained as over-the-counter drugs could not be obtained. However, most lipid-lowering agents require a prescription from a physician and cannot be purchased over the counter. Additionally, a few MACE may not have been captured despite the meticulous record review of our study population, which can limit our finding.

In conclusion, we demonstrated that the risk of long-term cardiovascular events in treatment-naive CHB patients treated with TAF and TDF were comparable. Distinct serial changes between the two treatments were shown in lipid profiles. Nevertheless, no significant difference in the TC/HDL ratio, which is thought to be well-associated with the risk of cardiovascular events, was observed between the two treatments. Further studies with longer follow-up periods are necessary to validate our findings.

**Authors’ contributions**

All authors had full access to all data used in this study and take responsibility for the data integrity and the accuracy of the data analyses. H Hong and J Choi were responsible for the conception and design of the study; W-M Choi, D Lee, JH Shim, KM Kim, Y-S Lim, and HC Lee were responsible for the acquisition, analysis, and interpretation of data as well as the drafting of the manuscript. J Choi performed the statistical analyses. All authors approved the final version of the manuscript.

**Acknowledgements**

This study was supported by grants from the National Research Foundation of Korea (NRF), funded by the Korean government (Ministry of Science and ICT) (No. 2021R1G1A1009506), Korea Health Technology R&D Project (No. HI21C2448), the Patient-Centered Clinical Research Coordinating Center (HC20C0062) of the National Evidence-based Healthcare Collaborating Agency, and the National R&D Program for Cancer Control through the National Cancer Center (HA21C0110), funded by the Ministry of Health and Welfare, Republic of Korea.

The authors would like to express their gratitude to Chae-yeon Lim and Mi Ryu for their assistance in this study.

**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**

cohort of patients with chronic hepatitis B. Aliment Pharmacol Ther 2020;52:371-381.


Artificial intelligence predicts direct-acting antivirals failure among hepatitis C virus patients: A nationwide hepatitis C virus registry program


1School of Medicine and Doctoral Program of Clinical and Experimental Medicine, College of Medicine and Center of Excellence for Metabolic Associated Fatty Liver Disease, National Sun Yat-sen University, Kaohsiung, Taiwan; 2Hepatobiliary Division, Department of Internal Medicine and Hepatitis Center, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 3Hepatitis Research Center, College of Medicine and Center for Liquid Biopsy and Cohort Research, Kaohsiung Medical University, Kaohsiung, Taiwan; 4Ph.D. Program in Translational Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, and Academia Sinica, Taipei, Taiwan; 5Division of Hepatogastroenterology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; 6Division of Gastroenterology and Hepatology, Department of Internal Medicine, E-Dea Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; 7School of Medicine for International Students, College of Medicine, I-Shou University, Kaohsiung, Taiwan; 8Division of Gastroenterology, Tainan Municipal Hospital (Managed By Show Chwan Medical Care Corporation), Tainan, Taiwan; 9Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chi Mei Medical Center, Yongkang District, Tainan, Taiwan; 10Department of Internal Medicine, Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Chiayi, Taiwan; 11School of Medicine, Tzu Chi University, Hualien, Taiwan; 12Division of Gastroenterology, Department of Internal Medicine, St. Martin De Porres Hospital, Chiayi, Taiwan; 13Division of Gastroenterology, Department of Internal Medicine, Taitung Mackay Memorial Hospital, Taitung, Taiwan; 14Mackay Medical College, New Taipei City, Taiwan; 15Division of Gastroenterology, Department of Internal Medicine, Yuan’s General Hospital, Kaohsiung, Taiwan; 16Division of Gastroenterology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; 17Division of Gastroenterology, Department of Internal Medicine, Tri-Service General Hospital Penghu Branch, National Defense Medical Center, Taipei, Taiwan; 18School of Medicine, Chung Shan Medical University, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan; 19Division of Gastroenterology, Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan; 20Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chi Mei Medical Center, Liouyong, Tainan, Taiwan; 21Lotung Poh-Ai Hospital, Yilan, Taiwan; 22Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; 23Institute of Clinical Medicine, School of Medicine, National Yang-Ming Chiao Tung University, Taipei, Taiwan; 24Division of Hepatology and Gastroenterology, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan; 25School of Medicine, Fu-Jen Catholic University, New Taipei City, Taiwan; 26Department of Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation and Tzu Chi University, Hualien, Taiwan; 27Department of Gastroenterology, Division of Internal Medicine, Show Chwan Memorial Hospital, Changhua, Taiwan; 28Division of Gastroenterology and Hepatology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; 29Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan; 30Liver Center, Cathay General Hospital, Taipei, Taiwan; 31Wen-Chih Wu Clinic, Fengshan, Kaohsiung, Taiwan; 32Division of Infectious Diseases, Department of
Approximately 1–3% of HCV patients experience DAA therapy failure. We conducted a nationwide study using AI to investigate the risk factors for DAA failure. The AI models outperformed the conventional logistic regression models. The AI model showed that subjects with features such as high HCV RNA levels, active hepatocellular carcinoma, or decompensated liver cirrhosis were prone to virological failure. Machine learning algorithms facilitate risk stratification in DAA failure and provide additional information on factors associated with DAA failure.
INTRODUCTION

Direct-acting antivirals (DAA) have changed the treatment landscape for patients infected with hepatitis C virus (HCV). However, despite the DAA efficacy being up to 97% across all HCV genotypes, approximately 1–3% of HCV patients fail to achieve a sustained virological response (SVR). Factors associated with DAA failure generally include decompensated liver cirrhosis, resistance-associated substitutions (RASs), the presence of hepatocellular carcinoma (HCC), prior treatment failures, and poor drug adherence. As comprehensive HCV elimination programs are advocated worldwide, an increasing number of patients with HCV infection are expected to require DAA salvage therapy. Thus, all the risk factors associ-
ated with DAA failure must be considered simultaneously to reduce the retreatment burden.

Artificial intelligence (AI) emerged as a powerful tool for disease diagnosis and risk assessment in healthcare. Factors contributing to treatment failure vary among individuals, making such heterogeneous data and complex interactions difficult to evaluate through regression methods. Moreover, conventional statistical methods can only handle linear data. Alternatively, machine-learning (ML) approaches can process both linear and nonlinear information and recognize the hidden relationships between variables and outcomes in big data. ML algorithms can be classified into supervised and unsupervised algorithms. Supervised ML is suitable for handling annotated data, whereas unsupervised ML can process datasets that lack class labels. Common supervised ML algorithms include decision trees (DT), random forest (RF), eXtreme Gradient Boosting (XGBoost), and artificial neural network (ANN). Thus, AI provides a new approach to understanding diseases by integrating multidimensional data with “automatic learning”. Advances in AI have made it possible to serve as a decision-support tool and improve diagnostic quality in healthcare.

We conducted a real-world, multicenter study using the Taiwan HCV Registry (TACR) database, aiming to explore the risk factors associated with DAA failure using artificial intelligence. We applied artificial intelligence to quickly distinguish HCV patients prone to virological failure.

**MATERIALS AND METHODS**

**Subjects**

The TACR Program is a nationwide HCV-registered platform implemented by the Taiwan Association for the Study of the Liver since 2020. The TACR conducted a real-world, multicenter, prospective cohort study of DAA therapy. A total of 34301 chronic hepatitis C patients >=18 years old who received DAAs with available SVR12 data were enrolled in this study. Patients with HCV who died during treatment or were lost to follow-up within 12 weeks after the completion of therapy were excluded from our study. The baseline demographics and virological characteristics before and after antiviral therapy were recorded in the TACR database. The primary outcome was the achievement of sustained virological response (SVR12), defined as undetectable HCV RNA in the serum after 12 weeks of end-of-treatment. The choice of antiviral regimens followed the international HCV treatment guidelines and the reimbursement criteria of the Taiwan National Health Insurance Administration. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital and adhered to the Declaration of Helsinki. Written informed consent was obtained from all the participants.

**Machine learning models**

The subjects were randomly assigned to a 70% training dataset (n=23,955) and a 30% validation dataset (n=10,346). Fifty-five host, virological, and on-treatment features were input into the ML models (Supplementary Table 1). The algorithms included DT, RF, XGBoost, and ANN. ML analysis was performed using the rpart, randomForest, xgboost, and neural network packages of R software. As some algorithms cannot handle missing data, missing data (14.3%) were imputed using the k-nearest neighbor method before generating predictions. The best model was used for risk stratification of patients with DAA treatment failure. Patients were further divided into subgroups using deciles of predicted risk probability to allow for more granular management of high-risk patients.

The performances of the ML models were assessed using the area under the receiver operating characteristic curve (AUROC), accuracy of the confusion matrix, precision-recall curve, and F1-score. A precision-recall curve closer to the upper-right corner indicates better performance. The F1-score is the weighted average of precision and recall and is favorable under class imbalance in the dataset. The F1-score ranged from 0 to 1; the predictive model with an F1-score closer to 1 is considered better. The Delong test was used to compare the differences in the AUC of the ROC curves. The codes for the ML models are presented in the Supplementary Materials.

**Statistical analyses**

Student’s t-test was used to compare continuous variables. Categorical variables were evaluated using the chi-square (X²) or Fisher’s exact test. Multivariate logistic regression analysis was performed to determine independent risk factors associ-
ated with treatment failure. Data were analyzed using the Statistical Package for the Social Sciences software (SPSS, version 26; IBM Co., Armonk, NY, USA). Statistical significance was defined as a two-tailed $P$-value $<0.05$.

**RESULTS**

**Baseline demographics**

The baseline demographics of the study participants are presented in Table 1, with no significant differences in age, sex, body mass index (BMI), biochemical data, cirrhosis, HCV genotypes, viral load, DAA regimens, HBV coinfection, or presence of HCC between the training and validation datasets.

**Logistic regression analysis of the factors associated with DAA treatment failure**

The overall DAA failure rate was 1.6%. In the univariate analysis, female, fibrosis-4 index (FIB-4), cirrhosis, decompensation, presence of HCC, HCV genotypes, higher HCV viral load, protease inhibitor-based DAA regimens, treatment experience, less DAA/ribavirin adherence, and severe adverse effects significantly increased the risk of DAA treatment failure. In biochemical examinations, lower albumin, platelet, and creatinine levels significantly increased the probability of non-SVR. Elevated aspartate aminotransferase (AST), bilirubin, prothrombin time, and hemoglobin A1c (HbA1c) levels significantly increase the likelihood of virological failure. In the multivariate analysis, the presence of cirrhosis and HCC, higher HbA1c, and less DAA adherence were independent risk factors for DAA treatment failure after adjustment for the variables with $P$-value $<0.05$ in the univariate analysis (Table 2).

We developed a conventional prediction model using logistic regression as follows:

Logistic regression (LR) model $= 3 \times \text{Liver cirrhosis (yes=1, no=0)} + 4 \times \text{HCC (yes=1, no=0)} + 1 \times \text{HbA1c} + 33 \times \text{DAA adherence (≤20%=5, 20–40%=4, 40–60%=3, 60–80%=2, >80%=1)}$

The components of the LR model were the four independent risk factors in the multivariate logistic regression analysis. The coefficient for each variable was derived from the odds ratio of the multivariate logistic regression analysis. The cutoff value for discriminating DAA failure was set at 40 using Youden’s index in the ROC curve analysis.

**Performance of the predictive models**

In the training dataset, the AUROC was 1.000, 1.000, 0.845, 0.736, and 0.588 for the XGBoost, random forest, decision tree, artificial neural network, and logistic regression models, respectively. The accuracy, precision, and recall rates of the prediction were 100% for both XGBoost and random forest. The F1 score achieved 1.00 in both the XGBoost and random forest algorithms (Fig. 1A, B, and Table 3).

In the validation dataset, the AUROC was 0.803, 0.756, 0.644, 0.658, and 0.616 for the XGBoost, random forest, decision tree, artificial neural network, and logistic regression models, respectively (Fig. 1C, D, and Table 3). The Delong test revealed the performance of XGBoost was superior to the random forest ($P=0.021$), decision tree ($P=4.4 \times 10^{-8}$), artificial neural network ($P=5.4 \times 10^{-8}$), and logistic regression model ($P=2.5 \times 10^{-16}$) (Table 4). The accuracy, precision, recall, and F1-score of XGBoost are 98.3%, 98.4%, 99.9%, and 0.992, respectively.

**Risk stratification based on the XGBoost algorithm**

The overall HCV patients receiving DAA treatment were further stratified according to the XGBoost prediction results. XGBoost provides a risk coefficient between 0 and 1 for each case. The higher the coefficient, the higher the chance of achieving SVR. DAA efficacy was divided into ten subgroups based on risk coefficient deciles. Figure 2 shows the predicted non-SVR accuracy for each subgroup using the XGBoost algorithm. The participants were stratified into high-risk (decile 1–5), intermediate-risk (decile 6–9), and low-risk (decile 10) populations based on the risk coefficients. The DAA failure rate was 75–100% in the high-risk, 15.8–40.0% in the intermediate-risk, and 0.4% in the low-risk populations. The DAA failure rate among the top five deciles was substantially higher than that at baseline (1.6%). The accumulative non-SVR rate was 69.7% in the high-risk population. Among the 538 subjects for whom DAA treatment failed, 375 (69.7%) were successfully detected using the XGBoost model among
Table 1. Baseline demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Training</th>
<th>Validation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>34,301 (100)</td>
<td>23,955 (70)</td>
<td>10,346 (30)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.9±12.7</td>
<td>61.9±12.6</td>
<td>61.9±12.8</td>
<td>0.710</td>
</tr>
<tr>
<td>Male (gender)</td>
<td>17,972 (52.4)</td>
<td>12,562 (52.4)</td>
<td>5,410 (23.3)</td>
<td>0.799</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7±4.04</td>
<td>24.7±4.06</td>
<td>24.7±4.01</td>
<td>0.285</td>
</tr>
<tr>
<td>Fibrosis index-4 (FIB-4)</td>
<td>3.31±3.37</td>
<td>3.30±3.37</td>
<td>3.33±3.36</td>
<td>0.438</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>7,051 (20.6)</td>
<td>4,903 (20.5)</td>
<td>2,148 (20.8)</td>
<td>0.542</td>
</tr>
<tr>
<td>Decompensated liver cirrhosis</td>
<td>932 (2.7)</td>
<td>637 (2.7)</td>
<td>295 (2.9)</td>
<td>0.316</td>
</tr>
<tr>
<td>HCC</td>
<td>2,822 (8.2)</td>
<td>1,967 (8.2)</td>
<td>855 (8.3)</td>
<td>0.874</td>
</tr>
<tr>
<td>Liver transplant</td>
<td>84 (0.2)</td>
<td>62 (0.3)</td>
<td>22 (0.2)</td>
<td>0.427</td>
</tr>
</tbody>
</table>

Genotype

1 16,638 (48.5) 11,577 (48.3) 5,061 (48.9) 0.924
2 13,298 (38.8) 9,332 (39.0) 3,966 (38.3)
3 543 (1.6) 381 (1.6) 162 (1.6)
4 17 (0.0) 13 (0.1) 4 (0.0)
5 6 (0.0) 5 (0.0) 1 (0.0)
6 2,478 (7.2) 1,730 (7.2) 748 (7.2)
Mixed 1,073 (3.1) 748 (3.1) 325 (3.1)
Unclassified 247 (0.7) 168 (0.7) 79 (0.8)

HCV RNA (log IU/mL) 5.90±1.02 5.90±1.02 5.90±1.02 0.623
HBsAg (+) 2,381 (7.3) 1,654 (7.3) 727 (7.4) 0.668
AST (IU/L) 60.7±54.1 60.4±53.9 61.3±54.5 0.165
ALT (IU/L) 73.0±77.0 72.8±77.7 73.3±75.4 0.635
Albumin (g/dL) 4.18±0.43 4.18±0.43 4.17±0.43 0.755
Total bilirubin (g/dL) 0.83±0.51 0.82±0.51 0.84±0.51 0.078
Platelet (x10³/μL) 181.4±72.1 181.8±72.3 180.5±71.5 0.122
Prothrombin time (INR) 1.05±0.31 1.05±0.30 1.05±0.34 0.174
HbA1c (%) 6.06±1.24 6.1±1.3 6.0±1.2 0.308

DAA regimens

Daclatasvir/Asunaprevir 981 (2.9) 682 (2.8) 299 (2.9) 0.986
Viekirax/Exviera 3,394 (9.9) 2,377 (9.9) 1,017 (9.8)
Elbasvir/grazoprevir 3,933 (11.5) 2,720 (11.4) 1,213 (11.7)
Ledipasvir/sofosbuvir 7,592 (22.1) 5,301 (22.1) 2,291 (22.1)
Sofosbuvir 2,549 (7.4) 1,770 (7.4) 779 (7.5)
Sofosbuvir/daclatasvir 726 (2.1) 512 (2.1) 214 (2.1)
Glecaprevir/pibrentasvir 7,568 (22.1) 5,300 (22.1) 2,268 (21.9)
Sofosbuvir/velpatasvir 7,415 (21.6) 5,196 (21.7) 2,219 (21.5)
Sofosbuvir/velpatasvir/voxilaprevir 90 (0.3) 60 (0.3) 30 (0.3)
Others 42 (0.1) 28 (0.1) 14 (0.1)
Ribavirin (+) 4,346 (12.7) 3,019 (12.6) 1,327 (12.8) 0.572
Treatment naive 29,540 (86.1) 20,616 (86.1) 8,924 (86.3) 0.663

Values are presented as number (%).
BMI, body mass index; FIB-4, fibrosis index-4; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, hemoglobin A1c; DAA, direct-acting antivirals.
Table 2. Multivariate logistic regression analysis of the factors associated with DAA treatment failure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVR</td>
<td>Non-SVR</td>
</tr>
<tr>
<td>Number (%)</td>
<td>33,763 (98.4)</td>
<td>538 (1.6)</td>
</tr>
<tr>
<td>Age</td>
<td>61.9±12.7</td>
<td>61.6±12.9</td>
</tr>
<tr>
<td>Male</td>
<td>17,722 (52.5)</td>
<td>250 (46.5)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.7±4.0</td>
<td>24.9±4.2</td>
</tr>
<tr>
<td>FIB-4</td>
<td>3.30±3.36</td>
<td>3.94±3.85</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>6,870 (20.4)</td>
<td>181 (33.6)</td>
</tr>
<tr>
<td>Decompensated LC</td>
<td>907 (2.7)</td>
<td>25 (4.6)</td>
</tr>
<tr>
<td>HCC</td>
<td>2,715 (8.0)</td>
<td>107 (19.9)</td>
</tr>
<tr>
<td>Liver transplant</td>
<td>82 (0.2)</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16,429 (48.7)</td>
<td>209 (38.8)</td>
</tr>
<tr>
<td>2</td>
<td>13,057 (38.7)</td>
<td>241 (44.8)</td>
</tr>
<tr>
<td>3</td>
<td>516 (1.5)</td>
<td>27 (5.0)</td>
</tr>
<tr>
<td>4</td>
<td>14 (0.0)</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>5</td>
<td>6 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>6</td>
<td>2,440 (7.2)</td>
<td>38 (7.1)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1,056 (3.1)</td>
<td>17 (3.2)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>244 (0.7)</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>HCV RNA (log IU/mL)</td>
<td>5.90±1.02</td>
<td>6.16±0.87</td>
</tr>
<tr>
<td>HBsAg (+)</td>
<td>2345 (7.3)</td>
<td>36 (7.0)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.18±0.43</td>
<td>4.07±0.51</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>60.6±54.1</td>
<td>67.4±54.2</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>72.9±77.1</td>
<td>76.0±73.6</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.83±0.51</td>
<td>0.90±0.55</td>
</tr>
<tr>
<td>Platelet (x10(^3)/μL)</td>
<td>181.6±72.0</td>
<td>169.4±74.9</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>1.05±0.31</td>
<td>1.08±0.44</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>1.17±1.54</td>
<td>1.05±1.30</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.1±1.2</td>
<td>6.3±1.5</td>
</tr>
<tr>
<td>DAA regimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daclatasvir/Asunaprevir</td>
<td>936 (2.8)</td>
<td>45 (8.4)</td>
</tr>
<tr>
<td>Viekirax/Exviera</td>
<td>3,353 (9.9)</td>
<td>41 (7.6)</td>
</tr>
<tr>
<td>Elbasvir/grazoprevir</td>
<td>3,888 (11.5)</td>
<td>45 (8.4)</td>
</tr>
<tr>
<td>Ledipasvir/sofosbuvir</td>
<td>7,470 (22.1)</td>
<td>122 (22.7)</td>
</tr>
<tr>
<td>Sofosbuvir</td>
<td>2,448 (7.3)</td>
<td>101 (18.8)</td>
</tr>
<tr>
<td>Sofosbuvir/daclatasvir</td>
<td>714 (2.1)</td>
<td>12 (2.2)</td>
</tr>
<tr>
<td>Glecaprevir/pibrentasvir</td>
<td>7,467 (22.1)</td>
<td>101 (18.8)</td>
</tr>
<tr>
<td>Sofosbuvir/velpatasvir</td>
<td>7,344 (21.8)</td>
<td>71 (13.2)</td>
</tr>
<tr>
<td>Sofosbuvir/velpatasvir/voxilaprevir</td>
<td>90 (0.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Others</td>
<td>42 (0.1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
the top five deciles (Fig. 2). When the cutoff value of the risk coefficient was set at 0.5, the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were 99.5%, 69.7%, 99.9%, 97.4%, and 99.5%, respectively (Supplementary Table 2).

Importance of predictors

The relative importance of DAA failure predictors was evaluated using the XGBoost algorithm in all cases. The x-axis represents the ratio of the number of times a variable is applied to the total number of trees. The top 12 predictors were body mass index, viral load, α-fetoprotein, bilirubin, platelets, FIB-4 index, creatinine, ALT, albumin, age, prothrombin time, and AST level (Supplementary Fig. 1).

SHAP summary plot

Shapley Additive exPlanations (SHAP) was used to separately measure the contributions to the outcome from each feature. Figure 3 shows the summary of the XGBoost model explainability with SHAP in all cases. SHAP>0 indicated a higher probability of SVR, while SHAP<0 indicated a higher chance of non-SVR. A dot represents a sample, and the colors represent feature values ranging from low (yellow) to high (purple). From the color distribution of the dots, we can deduce the effect of this feature on the DAA efficacy. For example, the purple dots for HCV RNA are concentrated at SHAP<0, indicating that a higher viral load increases the probability of treatment failure, whereas the yellow dots for albumin are concentrated at SHAP<0, indicating that subjects with low albumin levels are prone to treatment failure. In brief, elevated viral load, α-fetoprotein, FIB-4 index, bilirubin, and AST levels increase the risk of DAA failure. Subjects with a lower body mass index, platelets, albumin, and younger age had a lower probability of SVR.

The detailed relationships between the features and the SVR are shown in Figure 4, revealing nonlinear relationships between the predictors and DAA efficacy. For example, HCV RNA levels $<10^6$ IU/mL increased the likelihood of SVR, and HCV RNA $>2\times10^6$ IU/mL increased the risk of virological failure. Approximately, subjects aged $<60$ years, with serum bilirubin level $>2$ g/dL, albumin $<3.5$ g/dL, and creatinine level $>15$ mg/dL were prone to treatment failure. When AST ranges

---

Table 2. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SVR</td>
<td>Non-SVR</td>
</tr>
<tr>
<td>Treatment naive</td>
<td>29,113 (86.2)</td>
<td>427 (79.4)</td>
</tr>
<tr>
<td>DAA adherence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;80%</td>
<td>33,656 (99.7)</td>
<td>492 (91.8)</td>
</tr>
<tr>
<td>60-80%</td>
<td>44 (0.1)</td>
<td>10 (1.9)</td>
</tr>
<tr>
<td>40-60%</td>
<td>21 (0.1)</td>
<td>7 (1.3)</td>
</tr>
<tr>
<td>20-40%</td>
<td>19 (0.1)</td>
<td>11 (2.1)</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>5 (0.0)</td>
<td>16 (3.0)</td>
</tr>
<tr>
<td>Ribavirin adherence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;80%</td>
<td>3,910 (93.2)</td>
<td>117 (87.3)</td>
</tr>
<tr>
<td>60-80%</td>
<td>125 (3.0)</td>
<td>7 (5.2)</td>
</tr>
<tr>
<td>40-60%</td>
<td>77 (1.8)</td>
<td>6 (4.5)</td>
</tr>
<tr>
<td>20-40%</td>
<td>49 (1.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>35 (0.8)</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>Severe adverse effects</td>
<td>365 (1.3)</td>
<td>13 (2.9)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

DAA, direct-acting antivirals; BMI, body mass index; LC, liver cirrhosis; FIB-4, fibrosis index-4; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Cr, creatinine; Hba1c, hemoglobin A1c; adj. OR, adjusted odds ratio; CI, confidence interval.
between 200–300 IU/L, treatment failure is more likely; in contrast, AST <100 or AST >400 IU/L implies a higher chance of achieving SVR.

DISCUSSION

The nationwide TACR study investigated the risk factors for DAA treatment failure in Taiwanese patients. Multivariate regression analysis revealed that liver cirrhosis, HCC, poor DAA adherence, and high HbA1c levels were significantly associated with virologic failure. We developed an ML-based predictive model to identify potential treatment failure populations. The performance of the XGBoost model was superior to the other algorithms and the conventional logistic regression model. The AUROC of the XGBoost algorithm is 1.000 and 0.803 for the training and validation datasets, respectively. The AI predictive model successfully detected 69.7% of the subjects who failed to achieve SVR among the top five decile subgroups, thus implying that an AI-based model can effectively strengthen the decision-making process for antiviral therapy.

The AI model showed that subjects with features of liver cirrhosis prone to decompensation (i.e., higher FIB-4 index, bilirubin, and AST levels; lower albumin and platelets) were less likely to achieve SVR. Elevated AFP levels and decreased BMI (i.e., weight loss) are hallmarks of active HCC and predispose patients to treatment failure. In addition, HCV patients with a high baseline viral load had more difficulty in clearing the virus than those with a low viral load. These AI findings...
are consistent with those of the multivariate regression analysis. DAA adherence and HbA1c were not incorporated into the top 12 predictors in the AI model, possibly owing to collinearity between the above factors and other variables. Co-morbid diabetes in HCV-infected patients is well-known to increase the risk of HCC.\(^{16,17}\) A substantial proportion of decompensated patients who do not achieve SVR may experience adverse events or death-related early discontinuation.\(^{18}\) Body mass index had J- or U-shaped associations with overall and all-cancer mortality rates. Compared with healthy-weight individuals, life expectancy is shorter in underweight subjects.\(^{19,20}\) Liver cirrhosis and HCC were not classified as significant risk factors using the XGBoost algorithm. Liver cirrhosis vs. FIB-4 index or HCC vs. AFP levels showed a certain degree of collinearity. In contrast, the viral load was not an independent risk factor using multivariate logistic analysis but became a significant predictor using the XGBoost approach, possibly resulting from the appropriate cutoff value of HCV RNA not being embedded in the regression model. Previous studies have shown that the SVR12 rate significantly decreased in the high baseline viral load group compared to that in the low viral load group. However, the optimal cutoff values for high vs. low viral loads vary across studies.\(^{21,22}\) While pan-genotypic DAAs can be safely administered in traditionally difficult-to-treat HCV populations, managing patients with active HCC, decompensated liver cirrhosis, RASs,

### Table 3. Performance of the predictive models for the response of direct-acting antivirals

<table>
<thead>
<tr>
<th>Model</th>
<th>AUC (95% CI)</th>
<th>P-value</th>
<th>Accuracy</th>
<th>Precision</th>
<th>Recall</th>
<th>F1-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training dataset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XGBoost</td>
<td>1.000 (1.000–1.000)</td>
<td>2.2×10^{-238}</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>1.000</td>
</tr>
<tr>
<td>Random Forest</td>
<td>1.000 (1.000–1.000)</td>
<td>2.2×10^{-238}</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>1.000</td>
</tr>
<tr>
<td>Decision tree</td>
<td>0.845 (0.825–0.865)</td>
<td>1.1×10^{-134}</td>
<td>98.6%</td>
<td>98.6%</td>
<td>100%</td>
<td>0.993</td>
</tr>
<tr>
<td>Neural network</td>
<td>0.736 (0.711–0.762)</td>
<td>8.3×10^{-56}</td>
<td>98.5%</td>
<td>98.5%</td>
<td>100%</td>
<td>0.992</td>
</tr>
<tr>
<td>Logistic regression</td>
<td>0.588 (0.558–0.619)</td>
<td>1.3×10^{-4}</td>
<td>81.6%</td>
<td>98.7%</td>
<td>82.3%</td>
<td>0.898</td>
</tr>
<tr>
<td>Validation dataset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XGBoost</td>
<td>0.803 (0.769–0.837)</td>
<td>4.9×10^{-42}</td>
<td>98.3%</td>
<td>98.4%</td>
<td>99.9%</td>
<td>0.992</td>
</tr>
<tr>
<td>Random Forest</td>
<td>0.756 (0.722–0.790)</td>
<td>1.6×10^{-40}</td>
<td>98.4%</td>
<td>98.4%</td>
<td>100%</td>
<td>0.992</td>
</tr>
<tr>
<td>Decision tree</td>
<td>0.644 (0.594–0.695)</td>
<td>9.8×10^{-11}</td>
<td>98.2%</td>
<td>98.4%</td>
<td>99.8%</td>
<td>0.991</td>
</tr>
<tr>
<td>Neural network</td>
<td>0.658 (0.616–0.700)</td>
<td>1.6×10^{-12}</td>
<td>98.4%</td>
<td>98.4%</td>
<td>100%</td>
<td>0.992</td>
</tr>
<tr>
<td>Logistic regression</td>
<td>0.616 (0.571–0.662)</td>
<td>6.2×10^{-7}</td>
<td>82.1%</td>
<td>98.8%</td>
<td>82.8%</td>
<td>0.901</td>
</tr>
</tbody>
</table>

CI, confidence interval; XGBoost, eXtreme Gradient Boosting.

### Table 4. Delong test

<table>
<thead>
<tr>
<th>P-value (z-score)</th>
<th>Random Forest</th>
<th>Decision tree</th>
<th>Neural network</th>
<th>Logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XGBoost</td>
<td>1.000 (0.0)</td>
<td>&lt;0.0001 (15.5)</td>
<td>&lt;0.0001 (19.9)</td>
<td>&lt;0.0001 (26.5)</td>
</tr>
<tr>
<td>Random Forest</td>
<td>&lt;0.0001 (15.5)</td>
<td>&lt;0.0001 (19.9)</td>
<td>&lt;0.0001 (26.5)</td>
<td></td>
</tr>
<tr>
<td>Decision tree</td>
<td>&lt;0.0001 (8.0)</td>
<td>&lt;0.0001 (15.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neural network</td>
<td></td>
<td>&lt;0.0001 (8.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Validation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XGBoost</td>
<td>0.021 (2.3)</td>
<td>4.4×10^{-6}   (5.5)</td>
<td>5.4×10^{-7} (5.8)</td>
<td>2.5×10^{-10} (6.3)</td>
</tr>
<tr>
<td>Random Forest</td>
<td>3.9×10^{-5}   (4.1)</td>
<td>6.6×10^{-7} (5.0)</td>
<td>2.5×10^{-10} (5.6)</td>
<td></td>
</tr>
<tr>
<td>Decision tree</td>
<td>0.643 (–0.5)</td>
<td>0.354 (0.9)</td>
<td>0.102 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Neural network</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

XGBoost, eXtreme Gradient Boosting.
or prior DAA failure requires special attention. There are substantial amounts of nonlinear data in clinical practice that are difficult to evaluate using conventional statistical methods. The SHAP dependence plot showed the relationship between SVR and the predictors (Fig. 4). Clinicians can realize the optimal range of significant variables that contribute to SVR. The AI predictive model can assist in discriminating high-risk patients and alert clinicians to identify risk factors before initiating DAA therapy.

The advantages of ML include flexibility and scalability, making it preferable for processing nonlinear big data. The HCV-TARGET study in the United States and Europe (n=6,525) applied multiple algorithms (elastic net, neural network, random forest, and gradient boosting) to predict DAA treatment failure (C-index=0.64–0.69), superior to the multivariate logistic regression model (C-index=0.51). The HCV-TARGET study revealed that the top ten predictors were albumin, liver enzymes, bilirubin, sex, HCV RNA, sodium, HCC, platelet count, and tobacco use. The TACR and HCV-TARGET studies highlighted the vital roles of HCC and cirrhosis-related risk factors in DAA treatment failure. AI approaches confirmed the viewpoints of traditional statistics and further improved predictive performance compared with conventional statistical methods.

A meta-analysis revealed that patients with active HCC had a significantly lower SVR rate (73.1%) than those with inactive HCC (92.6%) or those without HCC (93.3%). The REAL-C study enrolled propensity score-matched HCV patients and confirmed that SVR rates were reduced in patients with active HCC but not those with inactive HCC (85.5% vs. 93.7%; P=0.03). Patients with active HCC may experience more adverse effects and early DAA discontinuation, which partially explains the suboptimal DAA response in this population. The mechanisms underlying suboptimal antiviral efficacy in patients with active HCC remain unclear, possibly attributed to the ineffective blood delivery of DAA to target sites and impairment of host immunity in HCC patients. HCC Patients with curative potential should be treated aggressively before
DAA administration to ensure a greater chance of viral clearance. However, the optimal timing of DAA initiation in patients with incurable HCC remains controversial. Viral eradication significantly reduces mortality in patients with HCC receiving either curative or palliative HCC therapy, thus suggesting that antiviral therapy should not hesitate on those subjects not eligible for curative HCC treatment.

Previous studies have reported that the SVR rate is lower in HCV patients with decompensated cirrhosis than in patients with compensated cirrhosis. The probability of achieving SVR varies based on the reserved liver function. Patients with higher Model for End-Stage Liver Disease (MELD) scores (>20, Child-Turcotte-Pugh class C) had lower SVR rates, more adverse effects, and a lower likelihood of liver function improvement. For patients with a MELD score ≥20, post-transplant HCV treatment is recommended, unless the expected waitlist time is more than six months. Patients with MELD scores <15 should be treated promptly. The grey zone of a MELD score, i.e., 15–19, requires tailored therapy on a case-by-case basis. Our study provided information to identify patients with high risk of treatment failure. Selection of proper DAA regimens with high efficacy and safety profiles and enhancing DAA adherence might help to ensure treatment efficacy.

XGBoost is a supervised ML algorithm under a gradient boosting framework. The ensemble method combines multiple models to produce more accurate predictions. Gradient boosting is an ensemble technique that corrects mistakes in

Figure 3. SHAP summary plot. The SHAP summary plot combined the feature importance and effects on DAA efficacy in all cases. The x-axis represents the SHAP value of the feature. A SHAP value >0 represents a positive correlation with SVR, and a SHAP value <0 represents a negative correlation with SVR. The overlapping points jittered along the x-axis represent the samples; the colors represent feature values ranging from yellow (low) to red (high). SHAP, Shapley additive explanations; DAA, direct-acting antivirals; SVR, sustained virological response; BMI, body mass index; AFP, α-fetoprotein; PLT, platelets; FIB-4, fibrosis-4 index; AST, aspartate aminotransferease; INR, international normalized ratio; APRI, aminotransferase to platelet ratio index.
Figure 4. SHAP dependence plot. SHAP dependence plot revealed that global model interpretations depend on the given features. SHAP, Shapley additive explanations; HCV, hepatitis C virus; BMI, body mass index; AFP, α-fetoprotein; PLT, platelets; FIB-4, fibrosis-4 index; AST, aspartate aminotransferase; INR, international normalized ratio; APRI, aminotransferase to platelet ratio index.
existing models by creating new models, which are sequentially added until no further improvement can be achieved.\textsuperscript{17} This process is called gradient boosting because it utilizes a gradient descent method to minimize loss when creating new models.\textsuperscript{38} Moreover, XGBoost supports both regression and classification tasks. XGBoost indeed exhibits an outstanding predictive ability compared to the other algorithms in our study.

ML models are usually considered “black boxes” because their prediction process is too complex for humans to interpret. To overcome this problem, explainable AI methods have been developed based on the Shapley methods.\textsuperscript{39} SHAP is derived from the concept of cooperative game theory, which can calculate the contribution of each feature to the prediction. SHAP provides insight into the inner workings of a “black box” by generating quantitative visualizations of the prediction process. SHAP can reflect the influence of the features in each sample and show a positive or negative impact on the outcome.\textsuperscript{15} Through the transformation of SHAP, users can better understand how the model makes predictions. Moreover, it provides feedback on the key factors contributing to the outcome and allows for the identification of potential biases. This transparency is crucial for convincing clinicians to rely on AI-based decision support systems.\textsuperscript{40} “Explainable AI” may help bridge the gap between the medicine and AI-predictive models.

The current study has several limitations. Some patients (e.g., hepatic decompensation, HCC) may not survive long enough to obtain SVR\textsubscript{12} data. This population may have relatively unfavorable prognostic factors, leading to a suboptimal treatment response. Although RASs have been confirmed to be associated with viral resistance,\textsuperscript{241} RAS testing is not recommended in routine clinical practice.\textsuperscript{42} Considering only 7.0\% of the RASs were available in the TACR database, this predictive model may underestimate the impact of RASs on the DAA response. The performance of the validation dataset is inferior to that of the training dataset. Potential overfitting and heterogeneity in the training dataset may affect model generalizability. However, avoiding overfitting may reduce the accuracy of ML models. In such an imbalanced dataset (non-SVR rate=1.6\%), we expect the accuracy of the training dataset to be at least >98.4\%. Under this premise, the hyperparameter tuning of each AI model was relatively limited, making it difficult to avoid overfitting. As the generalization is suboptimal, the present AI model should be further modified before being applied to other independent cohorts. A relatively small number of DAA failure events may limit the development of a robust model. There is no universal approach to missing data imputation—a fundamental concern in real-world clinical datasets. The input data for the AI analysis contained only clinical and virological data in the current study. A combination of genomics, proteomics, and metabolomics may improve the predictive accuracy of the validation datasets in the future.

In conclusion, this nationwide TACR study applied ML algorithms for risk stratification in DAA failure. The performance of the AI model is superior to that of the conventional logistic regression model. The XGBoost model showed that subjects with features such as higher HCV RNA levels, active HCC, or decompensated liver cirrhosis were less likely to achieve SVR. This model captured 69.7\% of patients who failed to achieve SVR among the top five decile subgroups. ML algorithms facilitate risk stratification in DAA failure and provide additional information on factors associated with DAA failure.

Authors’ contribution


Acknowledgements

This work was supported partially by the “Center For Intelligent Drug Systems and Smart Bio-devices (IDS2B)” and the “Center of Excellence for Metabolic Associated Fatty Liver Disease, National Sun Yat-sen University, Kaohsiung” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan, and grants from MOST 111-2314-B-037-069-MY2, MOHW111-TDU-B-221-114007, KMUH-DK(B)1111002-1, KMHK-DK(C)1111004, and KMHK-DK(C)1111006, KMU-TC111B04, KMU-TC111A04, NSTC 112-2321-B-001-006 and MOHW112-TDU-B-221-124007.
Conflicts of Interest

Ming-Lung Yu disclosed the following: research grant from Abbvie, Gilead, Merck, and Roche diagnostics; consultant for Abbvie, BMS, Gilead, Roche, and Roche diagnostics; and speaker for Abbvie, BMS, Eisai, Gilead, Roche, and Roche diagnostics.

SUPPLEMENTARY MATERIAL

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

REFERENCES

22. Salmon D, Bani-Sadr F, Gilbert C, Rosenthal E, Valantin MA,


Protein-centric omics analysis reveals circulating complements linked to non-viral liver diseases as potential therapeutic targets

Yingzhou Shi1,2,3,4,5,6,7, Hang Dong1,2,3,4,5,6,7, Shiwei Sun2,3,4,5,6,7, Xiaojin Wu8, Jiansong Fang9, Jianbo Zhao2,3,4,5,6,7,10, Junming Han2,3,4,5,6,7, Zongyue Li2,3,4,5,6,7, Huixiao Wu2,3,4,5,6,7, Luna Liu1,2,3,4,5,6,7, Wanhong Wu2,3,4,5,6,7, Yang Tian1,2,3,4,5,6,7, Guandou Yuan11, Xiude Fan2,3,4,5,6,7, and Chao Xu1,2,3,4,5,6,7

1Department of Endocrinology, Shandong Provincial Hospital, Shandong University, Jinan, Shandong, China; 2Key Laboratory of Endocrine Glucose & Lipids Metabolism and Brain Aging, Ministry of Education; Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China; 3Shandong Clinical Research Center of Diabetes and Metabolic Diseases, Jinan, Shandong, China; 4Shandong Institute of Endocrine and Metabolic Diseases, Jinan, Shandong, China; 5“Chuangxin China” Innovation Base of Stem Cell and Gene Therapy for Endocrine Metabolic diseases, Jinan, Shandong, China; 6Shandong Clinical Research Center of Diabetes and Metabolic Diseases, Jinan, Shandong, China; 7Shandong Engineering Laboratory of Prevention and Control for Endocrine and Metabolic Diseases, Jinan, Shandong, China; 8Northern Ohio Alcohol Center, Department of Inflammation and Immunity, Cleveland Clinic, Cleveland, OH, USA; 9Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China; 10Clinical Medical College, Ningxia Medical University, Yinchuan, Ningxia, China; 11Division of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

Graphical Abstract

Study Highlights

• Our study reveals a significant causal relationship between specific complement components and various non-viral liver diseases. Key findings include the association of C1QC with autoimmune hepatitis, CFHR5 with primary sclerosing cholangitis, and inverse correlations of CFHR1 and CFHR2 with alcohol-related cirrhosis. Additionally, several components like C15, C7, and CFHR2 show significant links to hepatocellular carcinoma. The identification of potential drugs for various liver diseases was achieved by network-based drug repositioning, using insights from the complement regulatory network.
INTRODUCTION
The proportion of non-viral liver diseases among chronic liver diseases (CLD) is increasing, resulting in serious disease and economic burdens.1 Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) are the most common non-viral liver diseases. The increasing incidence of these diseases is associated with aging and an aging population.2,3 The proportion of non-viral liver diseases among chronic liver diseases (CLD) is increasing, resulting in serious disease and economic burdens.1 Autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) are the most common non-viral liver diseases. The increasing incidence of these diseases is associated with aging and an aging population.2,3

Background/Aims: To evaluate the causal correlation between complement components and non-viral liver diseases and their potential use as druggable targets.

Methods: We conducted Mendelian randomization (MR) to assess the causal role of circulating complements in the risk of non-viral liver diseases. A complement-centric protein interaction network was constructed to explore biological functions and identify potential therapeutic options.

Results: In the MR analysis, genetically predicted levels of complement C1q C chain (C1QC) were positively associated with the risk of autoimmune hepatitis (odds ratio 1.125, 95% confidence interval 1.018–1.244), while complement factor H-related protein 5 (CFHR5) was positively associated with the risk of primary sclerosing cholangitis (PSC;1.193, 1.048–1.357). On the other hand, CFHR1 (0.621, 0.497–0.776) and CFHR2 (0.824, 0.703–0.965) were inversely associated with the risk of alcohol-related cirrhosis. There were also significant inverse associations between C8 gamma chain (C8G) and PSC (0.832, 0.707–0.979), as well as the risk of metabolic dysfunction-associated steatotic liver disease (1.167, 1.036–1.314). Additionally, C1S (0.111, 0.018–0.672), C7 (1.631, 1.190–2.236), and CFHR2 (1.279, 1.059–1.546) were significantly associated with the risk of hepatocellular carcinoma. Proteins from the complement regulatory networks and various liver disease-related proteins share common biological processes. Furthermore, potential therapeutic drugs for various liver diseases were identified through drug repurposing based on the complement regulatory network.

Conclusions: Our study suggests that certain complement components, including C1S, C1QC, CFHR1, CFHR2, CFHR5, C7, and C8G, might play a role in non-viral liver diseases and could be potential targets for drug development. (Clin Mol Hepatol 2024;30:80-97)

Keywords: Liver diseases; Complement system proteins; Mendelian randomization analysis; Drug repositioning

Abbreviations:
AIH, autoimmune hepatitis; ALC, alcohol-related cirrhosis; ALD, alcoholic liver disease; CFHR1, complement factor H-related protein 1; CFHR2, complement factor H-related protein 2; CFHR5, complement factor H-related protein 5; cis-pQTLs, cis-acting protein quantitative trait loci; CLD, chronic liver diseases; C1QC, C1Q subcomponent subunit; C15, Complement C1s ; C7, Complement C7; C8, complement C8; C8G, complement C8 gamma chain; GWAS, genome-wide association study; HCC, hepatocellular carcinoma; GTEx, Genotype-Tissue Expression; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes pathway; IVW, inverse-variance weighted; MAC, membrane attack complex; MR, mendelian randomization; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; TICs, tumor-initiating cells

Editor: Ju Dong Yang, Cedars-Sinai Medical Center, USA
Received: Sep. 3, 2023 / Revised: Dec. 4, 2023 / Accepted: Dec. 7, 2023
(PSC) represent the three major autoimmune liver diseases. In addition, metabolic dysfunction-associated steatotic liver disease (MASLD) and alcoholic liver disease are two other types of chronic liver disease. MASLD, formerly nonalcoholic fatty liver disease (NAFLD), has been redefined to encompass liver steatosis along with one or more of five specific cardiometabolic risk factors. This renaming and revised criteria reflect a deeper understanding of the disease's pathophysiology. Despite the change in name, MASLD continues to exhibit similar prevalence and risk factors as NAFLD, indicating a consistent approach to understanding and managing this liver condition. These non-viral liver diseases are anticipated to drive CLD epidemiology going forward and to account for increasing proportions of hepatocellular carcinoma (HCC) and death in the future. There is a lack of effective drugs for the treatment of these non-viral liver diseases, especially when the disease progresses to liver decompensation or HCC, at which point, liver transplantation may be the only effective treatment. Given the lack of effective therapies for non-viral liver diseases and the resulting increase in mortality, it is important to identify biomarkers associated with the occurrence and progression of non-viral liver diseases and to explore their potential as predictive targets for screening therapeutics.

The complement system, a critical component of the innate immune system, is activated by three independent pathways, namely classical, lectin, and alternative, involving more than 50 soluble and membrane-bound proteins. Complement was initially characterized as an essential system for protection against invading pathogens; however, more recent data highlight its role in uncontrolled inflammatory responses that contribute to the development of liver diseases, kidney diseases, tumors, and many other diseases. Most complement proteins are produced in the liver, before being released into the circulation and distributed throughout the body; consequently, changes in the complement system are closely associated with liver diseases. In view of this close association, complement components may be significantly related to the development of liver diseases, and it is of great significance to explore effective complement components as pharmacological intervention targets for the treatment of liver diseases. As the circulating complements are mainly produced by the liver, it is difficult to determine the causal association between the complement components and liver diseases. Whether specific complement components change with the development of liver disease or whether changes in the complement components trigger the occurrence of liver diseases warrants further exploration.

In this study, we developed a new method to investigate the potential causal relationships between complement components and various non-viral liver diseases.

Mendelian randomization (MR) is a powerful genetic epidemiology method that uses human genetics to infer causality. Estimates derived from MR analyses are less susceptible to bias from confounding and reverse causation. Employing MR, we delved into whether changes in these components are a cause or a consequence of risk of developing liver disease. In a pioneering step, we combined our MR findings with a network-based drug repositioning approach, diverging from traditional gene overlap analyses. This method not only identifies potential therapeutic drugs but also strengthens the validity of our MR results. Our study represents the first of its kind to apply MR techniques in evaluating the causal role of complement components in liver diseases and proposes a novel workflow that marries MR insights with network-based drug discovery. These efforts were directed towards filling the gap in effective treatments for non-viral liver diseases, with a particular focus on uncovering new therapeutic targets.

MATERIALS AND METHODS

Study design

We tested the association between circulating levels of complement proteins with five non-viral liver diseases and HCC using two-sample MR. Subsequently, based on the complement components determined by MR analysis, we constructed a complement protein-protein interaction network for each non-viral liver disease and HCC, explored its involvement in the biological processes of liver diseases, and conducted network-based drug repositioning. Figure 1 shows a schematic representation of the study design. We conducted this study in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology-MR criteria.

Complement instruments selection

Summary data from a proteomic genome-wide association
study (GWAS) including 35,559 Icelanders were used to identify cis-acting protein quantitative trait loci (cis-pQTLs) as MR instruments for circulating complements. All 35 complement genes involved in the GWAS were included in our study (Supplementary Table 1). Cis-pQTLs were defined as single-nucleotide polymorphisms (SNPs) located within 500 kb of the gene encoding the measured protein and associated with circulating complement at \( P < 5 \times 10^{-6} \). Linkage disequilibrium clumping was performed using the European reference population at an \( r^2 < 0.1 \) threshold (window: 10,000 kb). For certain complements (C1q Like 2, C2, Factor B, C1q Binding Protein, C1q Tumor Necrosis Factor [TNF]-Related Protein 1, and C1q Like 4), no significant cis-variants were identified at a threshold of \( P < 5 \times 10^{-6} \) (Supplementary Table 2). Therefore, these complements were excluded from further analysis.

**Description of study outcomes**

Genetic summary data for each non-viral liver disease were obtained from several large population-based cohorts and

---

**Figure 1.** Schematic of the study design. GWAS, genome-wide association study; MASLD, metabolic dysfunction-associated steatotic liver disease; AIH, autoimmune hepatitis; ALC, alcohol-related cirrhosis; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; HCC, hepatocellular carcinoma; cis-pQTLs, cis-acting protein quantitative trait loci; SNP, single nucleotide polymorphism.
case-control GWAS. These data sources are listed in Table 1. Detailed information on the summary data for the liver diseases is available in the Supplementary Materials.

Construction of protein-protein interaction network


Subsequently, we obtained validated complement component interacting proteins by selecting experimentally validated interacting proteins (Supplementary Table 3). Finally, we used the network extension method within the network topology association analysis (NTA), to obtain the top 20 ranking neighboring proteins based on each complement component and its interacting proteins, and constructed a protein interaction network. In the network extension method, random walk analysis was first used to rank all proteins in the selected network based on their network proximity to the input seeds (complement components and their interacting proteins) and then return an expanded subnetwork in which nodes are the input seeds and their top-ranking neighboring proteins and edges represent their relationship. In this study, we used the “WebGestaltR” R package (version 0.4.4) to conduct the NTA analysis.

Liver diseases-associated genes

In this study, we obtained liver disease-associated genes from the DisGeNET (https://www.disgenet.org/) and the DISEASES platform (http://diseases.jensenlab.org/). DisGeNET is a comprehensive discovery platform containing publicly available collections of genes associated with human diseases that integrates data from expert scientific literature, GWAS catalogs, curated repositories, and animal models. The DISEASES platform integrates gene-disease associations from automated text-mining of biomedical literature. We collected 322 alcohol-related cirrhosis (ALC)-associated, 63 MASLD-associated, 191 HCC-associated, 190 AIH-associated, 264 PSC-associated genes, from the DisGeNET and DISEASES platforms (Supplementary Table 4) (accessed in August, 2022).

Network-based drug repositioning

To identify potential therapeutic drugs for liver diseases, we applied a network proximity approach on the PharmOmics platform (http://mergeomics.research.idre.ucla.edu/runpharmomics.php) to compute the significance of the association between liver disease and drugs. PharmOmics introduces a precise transcriptomic knowledgebase and analytical instrument, enabling drug repositioning through specialized network methodologies for distinct species and tissues. Network-based drug repositioning is based on the connectivity in a given gene network between PharmOmics drug signatures and user input genes such as a disease signature. For each liver disease, the top 15 drugs were selected for pharmacovigilance analysis of their potential hepatotoxicity, and text mining of medical literature and clinical study registries was used to obtain information on the potential

Table 1. Sources of GWAS summary statistics for the analysis

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Total or Cases/Controls</th>
<th>Ancestry</th>
<th>Source (PMID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating complements components</td>
<td>35,559</td>
<td>European</td>
<td>34857953</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>821/484,413</td>
<td>European</td>
<td>34594039</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>121/456,227</td>
<td>European</td>
<td>34737426</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>4,796/19,955</td>
<td>European</td>
<td>27992413</td>
</tr>
<tr>
<td>Metabolic dysfunction-associated steatotic liver disease</td>
<td>1,483/17,781</td>
<td>European</td>
<td>32298765</td>
</tr>
<tr>
<td>Alcohol-related cirrhosis</td>
<td>712/1,466</td>
<td>European</td>
<td>26482880</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>123/456,225</td>
<td>European</td>
<td>34737426</td>
</tr>
</tbody>
</table>

GWAS, genome-wide association study.
therapeutic effects of the predicted drugs on liver disease. Comprehensive information about the construction of a protein-protein interaction network, acquisition of liver disease-associated genes, implementation of Gene Ontology (GO) enrichment analysis, identification of drugs with potential hepatotoxicity, and reference bibliographies for evidence of drug repurposing are included in the Supplementary Materials.

Bioinformatics analysis of the Genotype-Tissue Expression (GTEx) dataset

We utilized the GTEx Analysis V8 dataset, specifically the “GTEx_Analysis_2017-06-05_v8_RSEMv1.3.0_transcript.tpm.gct.gz” file, to explore the effect of individual complement components on the human liver. Our approach involved analyzing the raw RNA-seq gene “transcripts per million” data from the livers of 226 individuals. To compare liver gene expression, we categorized the expression levels of each complement component into four distinct quartiles. We then focused our analysis on contrasting the gene expression profiles between the highest 25% (upper quartile) and the lowest 25% (lower quartile) of complement component expression levels. This method allowed us to identify significant differences in liver gene expression associated with varying levels of specific complement components.

In our study, we identified differentially expressed genes for enrichment analysis using stringent criteria, specifically a False Discovery Rate (FDR) of < 0.05 and an absolute log Fold Change (|log FC|)≥1.5.

GO and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analysis

GO biological process and KEGG pathway enrichment analysis was performed using Metascape (https://metascape.org) on the liver diseases-associated genes, proteins from complement components-protein interaction network, and differentially expressed genes related to specific complement components (C1q C chain [C1QC], C1S, complement factor H-related protein [CFHR] 1, CFHR2, CFHR5, C7, and C8 gamma chain [C8G]). The GO biological process and KEGG pathway of co-enrichment of the aforementioned gene sets was demonstrated by heatmaps. This approach allowed us to juxtapose the enriched processes and pathways across the gene sets, emphasizing the specific roles and interactions of complement-related genes in liver diseases.

Statistical analyses

We performed 165 comparisons and applied a Bonferroni corrected significance level (P<0.05/165=0.0003). Analyses were conducted using the two-sample MR package (version 0.5.6) in R software (version 4.1.2). We use the inverse-variance weighted (IVW) approach as our primary analysis method. Further details regarding the MR methodology are provided in the Supplementary Materials.

Availability of data and materials

AIH, PBC, PSC, MASLD, and HCC GWAS summary statistics are publicly available at “https://www.ebi.ac.uk/gwas/” (last accessed on July 7, 2022) using PMIDs. Summary statistics for ALC are available at “http://gengastro.med.tu-dresden.de/suppl/alc_cirrhosis/”. Decoded proteomic GWAS summary data can be downloaded at “https://www.decode.com/summarydata/”. We have placed a larger table in the DataS1 file including instrument variables used in the MR analysis, complement components-protein interaction network, liver diseases-associated genes and pharmacovigilance studies using the FDA database.

RESULTS

Instrument characteristics

We obtained GWAS summary data for 35 complements from the deCODE study. A total of 28 complements remained after cis-pQTL definition screening and were included in the MR analysis (Table ). The F-statistics calculated for the instruments of complements analyzed in this study were >10, indicating a low susceptibility to weak instrument bias. The instrumental variables utilized in the MR analysis are listed in Supplementary Table 2.

AIH

Genetically predicted C1QC was nominally associated with AIH (IVW odds ratio [OR]: 1.125, 95% confidence interval [CI]
1.018–1.244; \( P=0.021 \) based on the cis-pQTL instrument definition (Fig. 2). However, this relationship was not robust in the weighted median (Fig. 3A, B). Based on the protein–protein interaction databases, we obtained a total of eight C1QC-interacting proteins, which were supported by experimental evidence. AIH-associated proteins and those from the C1QC regulatory network were found to be enriched in common biological processes primarily involving inflammation and immune-related processes (Fig. 4A). Figure 4B shows the top 15 potential therapeutic drugs obtained by drug repurposing based on the C1QC regulatory network. Among these drugs, 10 were found to have no hepatotoxicity (Supplementary Table 5), and eight (pioglitazone, medroxyprogesterone, dexamethasone, paricalcitol, losartan, estradiol, calcitriol, and rimonabant) have demonstrated therapeutic effects in various liver diseases in experimental studies. Furthermore, five drugs (pioglitazone, medroxyprogesterone, dexamethasone, calcitriol, and rimonabant) have demonstrated therapeutic effects against liver diseases in clinical studies. Previous studies have also highlighted the therapeutic effects of dexamethasone and calcitriol on AIH (Supplementary Table 6). 20-22

**PSC**

In accordance with the cis-pQTL definition, a nominally significant association was observed between genetically predicted C8G (IVW, OR: 0.832, 95% CI 0.707–0.979; \( P=0.027 \)) and CFHR5 (IVW, OR: 1.193, 95% CI 1.048–1.357; \( P=0.007 \)) in rela-

<table>
<thead>
<tr>
<th>Complement Instrument</th>
<th>Gene</th>
<th>Chr</th>
<th>Start</th>
<th>End</th>
<th>cis-pQTL (instrument)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement C1q and tumor necrosis factor-related protein 9A</td>
<td>CIQTNF9</td>
<td>13</td>
<td>24307166</td>
<td>24322535</td>
<td>22</td>
</tr>
<tr>
<td>Complement C1q subcomponent subunit C</td>
<td>C1QC</td>
<td>1</td>
<td>22643014</td>
<td>22648110</td>
<td>76</td>
</tr>
<tr>
<td>Complement C1q tumor necrosis factor-related protein 3</td>
<td>CIQTNF3</td>
<td>5</td>
<td>34017858</td>
<td>34042312</td>
<td>11</td>
</tr>
<tr>
<td>Complement C1q tumor necrosis factor-related protein 5</td>
<td>CIQTNF5</td>
<td>11</td>
<td>119338942</td>
<td>119340940</td>
<td>14</td>
</tr>
<tr>
<td>Complement Clr subcomponent</td>
<td>C1R</td>
<td>12</td>
<td>7080214</td>
<td>7092540</td>
<td>5</td>
</tr>
<tr>
<td>Complement Clr subcomponent-like protein</td>
<td>C1RL</td>
<td>12</td>
<td>7089587</td>
<td>7109238</td>
<td>5</td>
</tr>
<tr>
<td>Complement Cis subcomponent</td>
<td>C1S</td>
<td>12</td>
<td>6988259</td>
<td>7071032</td>
<td>7</td>
</tr>
<tr>
<td>Complement C3</td>
<td>C3</td>
<td>19</td>
<td>6677704</td>
<td>6730562</td>
<td>2</td>
</tr>
<tr>
<td>Complement C3b</td>
<td>C3</td>
<td>19</td>
<td>6677704</td>
<td>6730562</td>
<td>3</td>
</tr>
<tr>
<td>Complement C3b, inactivated</td>
<td>C3</td>
<td>19</td>
<td>6677704</td>
<td>6730562</td>
<td>2</td>
</tr>
<tr>
<td>Complement C3d fragment</td>
<td>C3</td>
<td>19</td>
<td>6677704</td>
<td>6730562</td>
<td>6</td>
</tr>
<tr>
<td>Complement C5</td>
<td>C5</td>
<td>9</td>
<td>120932642</td>
<td>121075195</td>
<td>15</td>
</tr>
<tr>
<td>Complement component C6</td>
<td>C6</td>
<td>5</td>
<td>41142116</td>
<td>41261348</td>
<td>18</td>
</tr>
<tr>
<td>Complement component C7</td>
<td>C7</td>
<td>5</td>
<td>40909492</td>
<td>40984643</td>
<td>50</td>
</tr>
<tr>
<td>Complement component C8 gamma chain</td>
<td>C8G</td>
<td>9</td>
<td>136945185</td>
<td>136946975</td>
<td>56</td>
</tr>
<tr>
<td>Complement component C9</td>
<td>C9</td>
<td>5</td>
<td>39284140</td>
<td>39371324</td>
<td>17</td>
</tr>
<tr>
<td>Complement decay-accelerating factor</td>
<td>CD55</td>
<td>1</td>
<td>207321519</td>
<td>207386804</td>
<td>27</td>
</tr>
<tr>
<td>Complement factor D</td>
<td>CFD</td>
<td>19</td>
<td>859453</td>
<td>867884</td>
<td>24</td>
</tr>
<tr>
<td>Complement factor H</td>
<td>CFH</td>
<td>1</td>
<td>196651754</td>
<td>196752476</td>
<td>18</td>
</tr>
<tr>
<td>Complement factor H-related protein 1</td>
<td>CFHR1</td>
<td>1</td>
<td>196819731</td>
<td>196832189</td>
<td>29</td>
</tr>
<tr>
<td>Complement factor H-related protein 2</td>
<td>CFHR2</td>
<td>1</td>
<td>196943738</td>
<td>196959622</td>
<td>38</td>
</tr>
<tr>
<td>Complement factor H-related protein 3</td>
<td>CFHR3</td>
<td>1</td>
<td>196774813</td>
<td>196795407</td>
<td>6</td>
</tr>
<tr>
<td>Complement factor H-related protein 4</td>
<td>CFHR4</td>
<td>1</td>
<td>196888014</td>
<td>196918713</td>
<td>34</td>
</tr>
<tr>
<td>Complement factor H-related protein 5</td>
<td>CFHR5</td>
<td>1</td>
<td>196977556</td>
<td>197009698</td>
<td>20</td>
</tr>
<tr>
<td>Complement factor I</td>
<td>CFI</td>
<td>4</td>
<td>109731008</td>
<td>109802150</td>
<td>21</td>
</tr>
<tr>
<td>Complement receptor type 1</td>
<td>CR1</td>
<td>1</td>
<td>207496147</td>
<td>207647656</td>
<td>41</td>
</tr>
<tr>
<td>Complement receptor type 2</td>
<td>CR2</td>
<td>1</td>
<td>207454230</td>
<td>207489895</td>
<td>31</td>
</tr>
</tbody>
</table>

Chr, chromosome; Cis-pQTLs, cis-acting protein quantitative trait loci; MR, mendelian randomization.
tion to the risk of PSC (Fig. 2). Although the results from the weighted median were not significant, they were consistent in direction (Fig. 3A, B). Based on protein–protein interaction databases, we identified 13 CFHR5–C8G-interacting proteins, which were supported by experimental evidence. PSC-associated proteins and those from the CFHR5–C8G regulatory network were found to be enriched in common biological processes, primarily involving the immune response, cell death, stress response, and other processes (Fig. 4C). Figure 4D shows the top 15 potential therapeutic drugs obtained by drug repurposing based on the CFHR5–C8G regulatory network. Among these drugs, 12 were found to have no hepatotoxicity (Supplementary Table 5), five (fluoxetine, mycophenolate, dexamethasone, calcitriol, and verteporfin) were found to have therapeutic effects on various liver diseases in experimental studies, and five (dexamethasone, iodine, calcitriol, mycophenolate, and atorvastatin) were found to have therapeutic effects on liver diseases in clinical studies; among these, three drugs (dexamethasone, mycophenolate, and atorvastatin) were found to have therapeutic effects on PSC in a previous study (Supplementary Table 7). 23,24

**PBC**

We observed no significant association between the 28 genetically predicted complement components and PBC using the IVW method (Fig. 2). Thus, our findings provide limited evidence to support a causal association between the complement system and PBC.

**MASLD**

Based on cis-pQTL instrument selection criteria, we observed a nominally significant association between genetically predicted concentrations of C8G and MASLD (IVW, OR: 1.167, 95% CI 1.036–1.314; \( P = 0.011 \), Fig. 2 and Fig. 3A, B). No
Figure 3. Consistent causal relationships and complement pathway visualization. (A) Consistent significant causal relationships estimated using different mendelian randomization (MR) approaches are depicted. Each point on the graph represents the odds ratio (OR) value, while the horizontal lines passing through the points indicate the corresponding 95% confidence interval. The robustness of the observed associations between circulating complement components and various non-viral liver diseases is reinforced by the consistent results across distinct MR techniques. (B) Visualization of the identified associations within the complement pathway, showcasing the dynamic interplay of circulating complement components linked to various non-viral liver diseases. The schematic representation provides an intuitive insight into the alterations of complement components associated with distinct liver diseases. This visualization enhances our understanding of the complex molecular relationships underlying the pathogenesis of non-viral liver diseases. AIH, autoimmune hepatitis; ALC, alcohol-related cirrhosis; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; MASLD, metabolic dysfunction-associated steatotic liver disease; HCC, hepatocellular carcinoma.
Figure 4. Network construction and drug repositioning for AIH, PSC, and MASLD. (A) The top 4 common enriched biological processes identified between the AIH-associated proteins and proteins from the C1QC regulatory network. (B) Sankey diagram illustrating the discovery process of potential therapeutic drugs for AIH based on the C1QC regulatory network through drug repurposing. (C) The top 5 common enriched biological processes identified between the PSC-associated proteins and proteins from the C8G-CFHR5 regulatory network. (D) Sankey diagram illustrating the discovery process of potential therapeutic drugs for PSC based on the C8G-CFHR5 regulatory network through drug repurposing. (E) The top 3 common enriched biological processes identified between the MASLD-associated proteins and proteins from the C8G regulatory network. (F) Sankey diagram illustrating the discovery process of potential therapeutic drugs for MASLD based on the C8G regulatory network through drug repurposing. GO, gene ontology; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; MASLD, metabolic dysfunction-associated steatotic liver disease; C1QC, complement C1q subcomponent subunit C; C8G, complement component C8 gamma chain; CFHR5, complement factor H-related protein 5.
pleiotropic effects were identified using the MR-PRESSO global test ($P=0.732$). However, this relationship did not survive the Bonferroni test for multiple corrections ($P<0.05/165=0.0003$). The top three biological processes enriched in proteins from the MASLD and C8G regulatory networks are shown in Figure 4E. Regulation of hormone levels (GO:0010817), carbohydrate metabolic processes (GO:0006109), and lipid metabolic processes (GO:0019216) were the three most common pathways. Among the 10 drugs without hepatotoxicity (Supplementary Table 5), seven

**Figure 5.** Network construction and drug repositioning for ALC and HCC. (A) The top 11 common enriched biological processes identified between the ALC-associated proteins and proteins from the CFHR1-CFHR2-C1QC regulatory network. (B) Sankey diagram illustrating the discovery process of potential therapeutic drugs based on the CFHR1-CFHR2-C1QC regulatory network through drug repurposing. (C) The top 5 common enriched biological processes identified between the HCC-associated proteins and proteins from the C1S-CFHR2-C7 regulatory network. (D) Sankey diagram illustrating the discovery process of potential therapeutic drugs based on the C1S-CFHR2-C7 regulatory network through drug repurposing. GO, gene ontology; ALC, alcohol-related cirrhosis; HCC, hepatocellular carcinoma; C1QC, complement C1q subcomponent subunit C; CFHR1, complement factor H-related protein 1; CFHR2, complement factor H-related protein 2; C1S, complement C1s subcomponent; C7, complement component 7.
Figure 6. Enrichment analysis of gene expression linked to complement components in liver diseases. (A) This heatmap offers a detailed analysis of GO biological process enrichment. It focuses on gene sets related to non-viral liver diseases and differential gene expression in the liver, in relation to several complement components (C1QC, C1S, CFHR1, CFHR2, CFHR5, C7, and C8G). The heatmap skilfully visualizes the top 20 enriched term clusters within the GO biological process category, derived from these specific gene sets. (B) This heatmap depicts KEGG pathway enrichment across various input gene lists. It is color-coded to represent $P$-values, displaying the top 20 clusters of KEGG pathway enrichment. Each cluster is marked by its most significantly enriched pathways, with a nuanced color gradient used to effectively convey the $P$-value significance.

GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; MASLD, metabolic dysfunction-associated steatotic liver disease; ALC, alcohol-related cirrhosis; HCC, hepatocellular carcinoma; C1QC, complement C1q subcomponent subunit C; C1S, complement C1s subcomponent; CFHR1, complement factor H-related protein 1; CFHR2, complement factor H-related protein 2; CFHR5, complement factor H-related protein 5; C7, complement component 7; C8G, complement component C8 gamma chain.
(imatinib, thalidomide, verteporfin, thrombin, dexamethasone, erlotinib, and vitamin A) were found to have therapeutic effects on MASLD, one (verteporfin) was found to have effects on HCC in experimental studies, and two (erlotinib and vitamin A) were found to have therapeutic effects on other liver diseases in clinical studies (Fig. 4F, Supplementary Table 8).^{25,26}

ALC

The genetically predicted CFHR1 concentration was inversely correlated with ALC after controlling for multiple testing (IVW, OR: 0.621, 95% CI 0.497–0.776; \(P=2.74\times10^{-5}\), Fig. 2); this association was consistent across sensitivity analyses (Fig. 3A, B). We also found nominally significant inverse associations between CFHR2 and ALC (IVW, OR: 0.824, 95% CI 0.703–0.965; \(P=0.017\)). MR-PRESSO revealed no evidence of horizontal or outlier variants in these relationships (Fig. 3A, B).

ALC-associated proteins and those from the CFHR1–CFHR2–C1QC regulatory network were found to be enriched in common biological processes primarily involving inflammation and immune-related processes (Fig. 5A). Figure 5B shows the top 15 potential therapeutic drugs obtained by drug repurposing based on the CFHR1–CFHR2–C1QC regulatory network. Among these drugs, four were found to have no hepatotoxicity (Supplementary Table S), four (lenalidomide, losartan, paricalcitol, and bortezomib) were found to have therapeutic effects on other liver diseases in experimental studies, and five (lenalidomide, losartan, decitabine, ascorbic acid, and thalidomide) were found to have therapeutic effects on ALC in a previous study (Supplementary Table 9).^{27,28}

HCC

Based on the cis-pQTL definition, CFHR2 (IVW, OR: 1.279, 95% CI 1.059–1.546; \(P=0.011\)) and C7 (IVW, OR: 1.631, 95% CI 1.190–2.236; \(P=0.002\)) were positively associated with HCC (Fig. 2 and Fig. 3A, B). HCC-associated proteins and those from the C1s–CFHR2–C7 regulatory network were found to be enriched in common biological processes, primarily immune response, cell activation, stress response, and other processes (Fig. 5C). Six drugs (docetaxel, melphalan, calcitriol, furosemide, thalidomide, and somatostatin) were found to have therapeutic effects on various liver diseases in clinical studies, of which four (docetaxel, melphalan, somatostatin, and thalidomide) were found to have therapeutic effects on HCC (Fig. 5D, Supplementary Table 10).^{29,30}

Role of complement components in liver disease pathogenesis

By individually analyzing the expression levels of C1QC, C1S, CFHR1, CFHR2, CFHR5, C7, and C8G, we identified distinct sets of differentially expressed genes for each complement component, totaling 429, 120, 304, 81, 178, and 53 genes, respectively (Supplementary Fig. 1 and 2). In our subsequent enrichment analysis of these genes in the context of nonviral liver diseases, we discovered a significant enrichment in biological processes primarily associated with inflammatory response, metabolic processes, and cell activation (Fig. 6A). Moreover, our analysis revealed that the enriched KEGG pathways predominantly involve complement pathways, drug metabolism, steroid hormone biosynthesis, and retinol metabolism (Fig. 6B). This comprehensive analysis underscores the multifaceted role of complement components in the pathophysiology of nonviral liver diseases, highlighting key areas for potential therapeutic intervention.

DISCUSSION

In this study, we used cis-MR analyses to evaluate the causal association between genetically predicted circulating levels of complements and non-viral liver diseases. Our results suggest a possible causal relationship between specific complement components and these liver diseases. For example, C1QC may be involved in the development of AIH and ALC. Additionally, C8G and CFHR5 may be associated with PSC, while C8G could potentially be linked to MASLD. Moreover, CFHR1 and CFHR2 might play a role in ALC, whereas C1S, C7, and CFHR2 may be associated with HCC. These findings provide insights into the potential involvement of the complement system in the development of these liver diseases. In addition, we identified several common pathways between proteins from the complement regulatory network and liver disease-related proteins, indicating potential interactions between the complement system and liver diseases. Furthermore, potential therapeutic drugs for various liver diseases...
have been identified through drug repurposing, utilizing the complement regulatory network.

C1QC was found to be related to the risk of AIH. C1QC, a component of the classical complement pathway, plays a crucial role in immune system regulation. A recent study has found a significant upregulation of C1QC in individuals with AIH when compared to control subjects, further emphasizing its potential importance in the pathogenesis of AIH. Additionally, recent research points to a significant association of C1QC with the progression of colon carcinoma and its involvement in skin cutaneous melanoma. Elevated expression of C1QC in colon neoplasms correlates with a more severe prognosis for affected patients. In a similar vein, C1QC has emerged as an independent predictive factor for the prognosis in cutaneous melanoma. Based on the regulatory network of C1QC, we identified a range of potential therapeutic agents for AIH including calcitriol, dexamethasone, medroxyprogesterone, pioglitazone and rimonabant. Notably, the use of calcitriol and dexamethasone is directly supported by experimental or clinical evidence for their effectiveness in AIH. Dexamethasone, a corticosteroid, is widely recognized as the standard treatment for AIH due to its potent anti-inflammatory and immunosuppressive effects. Calcitriol, the active form of vitamin D, has previously been recognized for its immunomodulatory properties, which may be pivotal in modulating the autoimmune response in AIH. Patients with chronic-onset AIH exhibit significantly lower vitamin D, underscoring the potential benefits of calcitriol supplementation.

We found that CFHR5 expression was positively associated with the risk of PSC, whereas C8G was inversely correlated. CFHR5, a member of the CFHR family, has previously been implicated in other autoimmune and inflammatory conditions, such as glomerulonephritis and systemic lupus erythematosus. Among the patients with glomerulonephritis studied, 12.6% (14 individuals) presented with eight distinct exonic variations in the CFHR5 gene. Notably, these patients had lower serum CFHR5-5 levels than the control participants. Its role in the complement pathway, which is integral to the innate immune response, suggests a potential mechanism through which it could influence the pathogenesis of PSC. On the other hand, the inverse relationship observed between C8G and PSC risk is equally noteworthy. C8G is one of the three subunits of C8. A study has identified C8G as a potential inhibitor of neuroinflammation. Moreover, the evidence suggests a vital role for C8G in astrocytes for the preservation of the blood-brain barrier during instances of inflammation within the brain. This anti-inflammatory property provides insights into its potential as drug target in PSC. In the drug repurposing analysis, we identified dexamethasone, atorvastatin, calcitriol, iodine, mycophenolate as potential therapeutic agents based on C8G-CFHR5 regulatory network. Among these drugs, dexamethasone and calcitriol show stronger evidence for potential effect in PSC. Specifically, a cohort study of 21 patients treated with 9 mg of budesonide, a corticosteroid like dexamethasone, for 1 year showed an improvement in portal inflammation and a notable reduction in serum alkaline phosphatase and aspartate transaminase levels compared to their baseline. Additionally, in a cholestasis mouse model, the intervention of calcitriol significantly modified gene expression associated with liver bile acid synthesis and transportation, as well as the mRNA expression of proinflammatory cytokines.

We found a nominally significant association between the circulating concentrations of C8G and MASLD. Intriguingly, this association was inverted in cases of PSC. MASLD is marked by the accumulation of fat in the liver, whereas PSC is a chronic condition characterized by inflammation and scarring of the bile ducts. This suggests that C8G might play varied roles in these two distinct liver conditions. Moreover, 6 drugs in our drug repositioning analysis showed potential therapeutic effects for MASLD, as supported by existing literature. Vitamin A has shown potential in managing MASLD by influencing lipid metabolism and reducing liver inflammation. On the other hand, Veraporfin, known for its use in photodynamic therapy, might offer benefits in MASLD treatment by targeting pathways involved in liver fibrosis, a complication of the disease. The genetically proxied concentration of CFHR1 and CFHR2 have shown an inverse association with ALC. CFHR1 and CFHR2 belongs to the factor H protein family, which plays a role in regulating innate immune complement reactions, particularly in preventing complement activation by inhibiting C5 convertase and terminal complement complex formation. The activation of C5 contributes to ethanol-induced fatty liver in mice by increasing inflammation. Therefore, it is conceivable that increased circulating concentrations of CFHR1 and CFHR2 may prevent people from developing ALC by inhibiting complement activation. Furthermore, studies have demonstrated that CFHR1 has relevance in malignan-
cies such as lung adenocarcinoma and myelogenous leukemia.\textsuperscript{40,41} Specifically, lung adenocarcinoma samples exhibit low levels of CFHR1 and deletion of both alleles of the CFHR1 gene has been identified as a potential predictor for the risk of developing acute myelogenous leukemia. Additionally, our results indicate an association between C1QC and ALC risk. Studies have reported that C1Q-deficient mice displayed a notable resistance to ethanol-induced liver damage, underscored by a decrease in hepatic triglyceride accumulation and a reduction in hepatocellular apoptosis.\textsuperscript{42} In our drug repurposing study centered on the CFHR1-CFHR2-C1QC regulatory network, we identified four potential drugs: lenalidomide, losartan, ascorbic acid, and thalidomide. Notably, thalidomide has been reported to prevent alcoholic liver injury in rats\textsuperscript{27} and effectively lower the hepatic venous pressure gradient in patients with stable alcoholic cirrhosis, owing to its ability to inhibit the production of TNF-alpha production.\textsuperscript{28}

We also found that C7 was associated with an increased risk of HCC. C7 plays a crucial role in the lytic pathway of the complement system, leading to the formation of the membrane attack complex. C7 has been recently acknowledged as a novel risk gene for Alzheimer’s disease in the Han Chinese population, with the p.K420Q mutation associated with disrupted immune activation and consequent changes in cerebral architecture and functionality.\textsuperscript{43} Additionally, C7 is found to be required to maintain the stemness of liver cancer cells. Consistent with our findings, C7 has been previously reported to be upregulated in liver tumor-initiating cells, and depletion of C7 has been found to abrogate the formation of tumor spheres.\textsuperscript{44} CFHR2 was found to be associated with HCC. In contrast, a negative correlation was observed between C1S and HCC. C1S is a component of C1, which serves as the primary component of the classical pathway in the complement system. Interestingly, 8 of the 11 drugs without hepatotoxicity were found to have evidence of therapeutic effects on HCC in C1S-CFHR2-C7 network-based drug repurposing. Docetaxel, melphalan, somatostatin, and thalidomide have demonstrated potential therapeutic efficacy against HCC in both basic research and preclinical studies. For example, the results of a phase II trial of thalidomide indicated that it can provide disease stabilization and improve survival rates in patients with HCC.\textsuperscript{29} Moreover, a recent study showed that thalidomide inhibits angiogenesis induced by chemotherapy-injured HCC cells.\textsuperscript{30}

This is the first study using MR techniques to evaluate whether complement components play a causal role in several liver diseases. A strength of our study was that we used variants in close proximity to the encoding gene to minimize horizontal pleiotropy. Pleiotropy observed in cis-SNPs tends to be vertical rather than horizontal, whereas vertical pleiotropy does not violate MR assumptions.\textsuperscript{45} To ensure adequate statistical power for our MR analysis, we used summary data from the largest available liver disease GWAS database. We also employed network-based drug repositioning to identify potential therapeutic drugs, which demonstrated stronger and more robust performance than gene overlap-based analysis. Furthermore, we proposed a novel workflow that combines MR findings with network-based drug repositioning to identify potential drugs and confirm the rationality of the MR results.

Our study had several limitations that warrant discussion. After multiple-testing corrections, most of the associations were not preserved in our MR analysis; therefore, the results need to be validated in a GWAS with a larger sample size. Moreover, to reduce the potential population stratification bias, we restricted the GWAS summary datasets to individuals of European ancestry. Hence, the results may not be generalizable to individuals with other ancestries. Despite uncovering substantial experimental and clinical evidence supporting the predicted drugs, there is a pressing need for more extensive research to thoroughly validate these findings. To unlock the full potential of these identified drugs for novel therapeutic applications, a cohesive and efficient research strategy is essential. This should start with detailed preclinical studies to elucidate the drugs’ mechanisms and identify any potential adverse effects. Following this, initial clinical trials are crucial to establish safety profiles and optimal dosages. Once these foundational aspects are in place, it becomes imperative to conduct larger-scale clinical trials encompassing a varied patient demographic to ensure a comprehensive evaluation of the drugs’ effectiveness. Such a methodical approach is vital for advancing these drugs from promising candidates to viable medical treatments.

In summary, our study provides evidence supporting a causal link between various complement components and liver diseases. These findings suggest that circulating complement components contribute to the pathophysiology of liver diseases and highlight their potential usefulness in drug repositioning.
Authors’ contribution
Conceptualization: CX, XF, GY, YS, XW. Data curation: CX, XF, GY, YS, HD, SS. Formal analysis: XF, YS, JF, JZ, ZL, LW. Funding acquisition: CX, XF, GY. Investigation: YS, HD, ZL, HW, LL, YT. Methodology: CX, XF, GY, YS, JF, JH. Project administration: CX, XF, GY, YS. Resources: XF, YS, HD, SS, XW. Supervision: CX, XF, GY, YS. Validation: CX, XF, GY, YS, HD, SS. Visualization: CX, XF, GY, YS, HD, SS, XW, JF, JZ. Writing – original draft: XF, YS, HD. Writing – reviewing and editing: CX, XF, GY, YS, HW, WW, YT. All the authors approved this version of the manuscript to be published.

Acknowledgements
The scientific calculations in this paper have been done on the HPC Cloud Platform of Shandong University. This work was supported by the National Natural Science Foundation (Grants No. 82200659), and the Natural Science Foundation of Shandong Province (Grant No. ZR20220H002).

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
44. Seol HS, Lee SE, Song JS, Rhee JK, Singh SR, Chang S, et al. Complement proteins C7 and CFH control the stemness of liver
Hepatitis B core-related antigen dynamics and risk of subsequent clinical relapses after nucleos(t)ide analog cessation

Ying-Nan Tsai, Jia-Ling Wu, Cheng-Hao Tseng, Tzu-Haw Chen, Yi-Ling Wu, Chieh-Chang Chen, Yu-Jen Fang, Tseng-Huey Yang, Mindie H. Nguyen, Jaw-Town Lin, and Yao-Chun Hsu

1Division of Gastroenterology and Hepatology, E-Da Cancer Hospital, I-Shou University, Kaohsiung, Taiwan; 2School of Medicine, College of Medicine, I-Shou University, Kaohsiung, Taiwan; 3Department of Public Health, National Cheng Kung University, College of Medicine, Tainan, Taiwan; 4Division of Gastroenterology and Hepatology, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; 5Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 6Department of Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 7Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, Yun-Lin, Taiwan; 8Division of Gastroenterology, Department of Medicine, Lotung Poh-Ai Hospital, Yilan, Taiwan; 9Division of Gastroenterology and Hepatology, Stanford University Medical Center, Palo Alto, CA, USA; 10Department of Epidemiology and Population Health, Stanford University Medical Center, Palo Alto, CA, USA

Study Highlights

- Posttreatment HBcrAg levels more accurately predict clinical relapse following discontinuation of entecavir or tenofovir than end-of-treatment HBcrAg and dynamic HBsAg levels do
- An HBcrAg level below 1,000 U/mL during the posttreatment follow-up indicates a low risk of subsequent clinical relapse
- Changes in HBcrAg levels after treatment in CHB patients are typically minor and the patterns of changes are not independently linked to clinical relapse risk.
INTRODUCTION

Nucleos(t)ide analogs (NA) suppress the replication of hepatitis B virus (HBV) by inhibiting the viral reverse transcriptase, thereby reducing hepatic inflammation, other liver-related comorbidities, and hepatocellular carcinoma (HCC).\(^1\)\(^-\)\(^3\) However, the ideal treatment endpoint, such as hepatitis B surface antigen (HBsAg) seroclearance, is rarely achieved during NA treatment.\(^4\)\(^-\)\(^5\) Most patients who remain HBsAg-positive experienced viral relapse after stopping NA. Hepatitis flares can occur following viral relapse, and severe acute exacerbations can lead to fatal consequences in some patients.\(^6\)\(^-\)\(^7\) Nevertheless, a limited number of studies have reported high HBsAg seroclearance rates after cessation of NA\(^8\)\(^,\)\(^9\) and ex vivo studies also indicate that HBV-specific T cell responses could be enhanced by discontinuing treatment with viral suppressants.\(^10\)\(^,\)\(^11\) Thus, the identification of groups of patients with chronic hepatitis B (CHB) who need to maintain NA or who can safely discontinue NA is an important yet unresolved issue.

HBcrAg is a potentially novel and useful biomarker that includes the hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), and a truncated precore protein (p22Cr).\(^12\) Serum HBcrAg level correlates with nuclear cccDNA activity, and could be used to predict HBeAg seroconversion, sustained treatment response to NA, and the risk of develop-
ment or recurrence of HCC. Prior studies have demonstrated that serum HBcrAg level at the time of NA cessation is useful for predicting off-therapy relapse and identifying patients for whom NA discontinuation may be potentially suitable. However, the levels of HBcrAg can change after cessation of treatment, and it is unclear whether dynamic measurements of HBcrAg off-therapy may better predict subsequent CR than the fixed HBcrAg level measured at end of treatment (EOT). Furthermore, no studies have yet compared the kinetics of HBV viral markers post-treatment to their association with clinical relapse risk following NA discontinuation. To address this knowledge gap, our study retrospectively analyzed data and biospecimens from a multicenter cohort of prospectively enrolled patients, serially measuring HBcrAg, HBsAg, and HBV DNA levels after therapy cessation. Our aim was to elucidate the relationship between the dynamic changes in these markers off-therapy and the subsequent CR after stopping NA.

MATERIALS AND METHODS

Study design and setting

We retrospectively analyzed data and assayed serum collected from a multicenter cohort of patients with CHB who were prospectively followed up after discontinuation of NA treatment. The protocol was previously reported in detail. Patients were prospectively enrolled from the E-Da Hospital (Kaohsiung, Taiwan), the Lotung Poh-Ai Hospital (Yilan, Taiwan), and the National Taiwan University Hospital Yun-Lin Branch (Yunlin, Taiwan) between July 1, 2011 and January 31, 2022. All participants provided signed informed consent before enrollment and agreed to the storage of their blood samples for subsequent studies. The present study was approved by the institutional review boards of the E-Da Health-care System (EMRP-110-100).

Study participants

Patients older than twenty years who had been diagnosed with CHB for longer than six months before treatment, who had continuously received either entecavir or tenofovir for at least two years, and who had undetectable HBV DNA and negative HBeAg at EOT were eligible for this analysis. Patients with detectable HBV DNA or positive HBeAg at discontinuation of NA, liver cirrhosis diagnosed either by histology or clinical features, co-infection with hepatitis C virus or human immunodeficiency virus, ascites, hepatic encephalopathy, variceal bleeding, malignancy, those who were organ transplant recipients, or who had received cytotoxic or immunosuppressive agents were excluded.

Patients enrolled in this study had to discontinue NA in accordance with the national health insurance policy of Taiwan. Details of the reimbursement rules were previously reported. Briefly, patients who were HBeAg–negative before treatment had to discontinue antiviral therapy after a maximum treatment duration of three years, but patients who were HBeAg–positive before treatment could maintain antiviral therapy until one year after HBeAg seroconversion. Patients could opt to pay for NA therapy themselves when their treatment period ended.

Laboratory examinations, including quantification of serum HBcrAg

The study baseline measurements at cessation of NA included the patients’ demographic, biochemical, serological, and virological data. Quantification of HBV DNA was performed using polymerase chain reaction (COBAS TaqMan HBV Test, version 2.0; Roche Molecular Systems, Inc., Alameda, CA, USA); the range of detection was 20 to 1.7×10^8 IU/mL. Serum levels of HBsAg were quantified using a micro–particle immunoassay (Abbott Architect i2000; Abbott Laboratories, Abbott Park, IL, USA). The upper limit of automated quantification was 250 IU/mL. Serum samples obtained at EOT, one and two years after treatment cessation that had been stored at −80°C, were used to analyze the dynamic changes in HBcrAg. Serum HBcrAg was quantified using the Lumipulse G HBcrAg assay on a Lumipulse G1200 Analyzer (Fujirebio Inc., Sagamihara Facility, Japan). The detection range varied from 1,000 to 10^7 U/mL. Samples with either HBsAg or HBcrAg levels higher than the upper limits of automatic detection were manually diluted to enable precise quantification.

Follow-up and definitions of outcomes after cessation of NA

After discontinuation of NA, patients were followed up ev-
ever three months. Follow-up included a physical health check and blood examinations, such as serum biochemistry, HBeAg, antibody to hepatitis B e antigen (anti-HBe), and HBV DNA, depending on the patient’s clinical situation at the time of the visit. Patients also underwent an abdominal ultrasound and serum alpha-fetoprotein tests every six months for HCC surveillance. Occurrence of CR was the primary study outcome and was defined as elevation of serum alanine transaminase (ALT) two times above the upper limit of the normal range (ULN) plus serum HBV DNA higher than 2,000 IU/mL. The ULN of serum was set at 40 U/L in accordance with the Asian-Pacific guidelines.21 The clinical outcomes of all patients were monitored until they restarted antiviral treatment, were lost to follow-up, or after January 31, 2022. The annual measurement of serum HBcrAg and HBsAg continued until either the occurrence of a CR or the end of the study. The temporal relationship between repeated measurement of serum HBcrAg levels and CR are shown in Supplementary Figure 1. According to the national health insurance policy in Taiwan, patients did not restart antiviral therapy for either virological relapses or transient hepatitis flares. NA was resumed for persistent hepatitis, defined as an elevation of serum ALT two times above ULN for at least three months and concerns of hepatic decompensation, with serum total bilirubin >2 mg/dL or prolonged prothrombin time >3 seconds.

**Statistical analyses**

Descriptive results for categorical and continuous variables are expressed as exact numbers with percentages and medians with interquartile ranges (IQR), respectively.

HBcrAg was imputed as 500 U/mL for statistical analysis if serum HBcrAg level was lower than 1,000 U/mL. Serum levels of HBsAg (IU/mL) and HBcrAg (U/mL) were transformed logarithmically for analysis. HBcrAg levels were also analyzed as a categorical variable above or below 1,000 U/mL. The cumulative incidences of CR were estimated by the Kaplan–Meier method and comparisons among different patient subgroups were performed using the log–rank test. Cox proportional hazard regression was used to identify potential risk factors. We analyzed the dynamic changes in HBcrAg, HBsAg, and HBV DNA levels at the EOT, and one and two years post-treatment cessation, as time-varying variables. Time-dependent models were employed to assess the association of these viral markers’ changes over time with the risk of CR. We applied the stepwise changes approach to eliminate factors that were not statistically significant during variable selection. For comparative purposes, however, we deliberately included the EOT levels of HBcrAg and HBsAg, as well as their time-varying levels, in the multivariable model, irrespective of their statistical significance. Hazard ratios (HR) were reported along with a 95% confidence interval (CI). All statistical examinations were two sided and P-values less than 0.05 were defined as statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Co., Armonk, NY, USA), except for time-dependent Cox regression model which was generated using R (version 4.1.3; R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Baseline characteristics of the study participants**

This study included 156 male (76.8%) and 47 female (23.2%) patients with CHB who discontinued NA, with the median age of 49.8 years (IQR, 41.9–59.0). Most patients (n=123, 60.6%) received entecavir and the median duration of treatment was 36.9 months (IQR, 36.5–40.1). The EOT serum HBsAg was 2.7 (IQR, 2.0–3.0) log IU/mL and the EOT serum HBcrAg was 3.0 (IQR, 2.0–3.9) log U/mL, respectively. The median follow-up time was 31.7 months (IQR, 16.7–67.1) (Table 1).

**CR after discontinuation of NA and association with EOT HBcrAg level**

During the follow-up period, CR occurred in 104 patients with a cumulative incidence of 49.5% (95% CI, 42.2–56.7%), 54.8% (95% CI, 47.1–62.4%) and 58.2% (95% CI, 50.1–66.0%) at 3, 5, and 7 years, respectively (Fig. 1). Eight patients experienced hyperbilirubinemia with serum total bilirubin over 2 mg/dL after CR, but recovered fully after resumption of NA therapy.

The lower limit of HBcrAg detection in the current study was 1,000 U/mL. This value has been used as the cutoff as suggested by the Japan Society of Hepatology (JSH).22 The
incidence of CR was significantly higher with the EOT HBcrAg ≥1,000 U/mL than <1,000 U/mL ($P=0.002$) (Fig. 2). In the univariate analysis, the HR for CR was 1.30 (95% CI, 1.10–1.53) per log U/mL ($P=0.005$) for the EOT HBcrAg (Table 2). In the receiver operating characteristic curve designed to evaluate the performance of EOT HBcrAg, however, the EOT HBcrAg level alone could not sufficiently predict the risk of CR, with an AUC of 0.61 (95% CI, 0.53–0.69) (Fig. 3).

Changes in serum HBcrAg in post-treatment monitoring and the associations with CR

After stopping treatment, 114 and 71 patients remained off NA after the first and second years. Their posttreatment fluctuations in HBcrAg levels were illustrated in relation to the occurrence of CR (Fig. 4). At treatment cessation (Fig. 4A), 59.6% (n=59) of patients without CR (n=99) had HBcrAg levels below 3 log U/mL, compared to 37.5% (n=39) of patients with CR (n=104). At one year post NA cessation, these figures were 67.1% (n=53 out of 79) and 54.3% (n=19 of 35), respectively (Fig. 4B), and the corresponding figures at two years were 68.4% (n=39 of 57) and 35.7% (n=5 of 14), respectively (Fig. 4C). On the other hand, a higher proportion with HBcrAg levels above 4 log U/mL was consistently observed in patients with CR at all time points: 29.8% (n=31 of 104) at cessation, 11.4% (n=4 of 35) at one year, and 14.3% (n=2 of 14) at two years, compared to 18.2% (n=18 of 99), 6.3% (n=5 of 79), and 5.3% (n=3 of 57), respectively for those without CR.

The proportions of patients with different HBV DNA and HBsAg levels in relation to subsequent CR at various time points were illustrated in the form of Kaplan-Meier curves. Figure 1 shows the cumulative incidence of clinical relapse following discontinuation of nucleos(t)ide analogues in the study population. Figure 2 illustrates the cumulative incidence of clinical relapse according to serum level of hepatitis B core–related antigen measured at the end of treatment. EOT, end-of-treatment; HBcrAg, hepatitis B core–related antigen.

**Table 1.** Baseline characteristics of the study patients

<table>
<thead>
<tr>
<th>Characteristics*</th>
<th>All patients (n=203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>49.8 (41.9–59.0)</td>
</tr>
<tr>
<td>Male sex</td>
<td>156 (76.8)</td>
</tr>
<tr>
<td>Positive anti-HBe</td>
<td>192 (94.6)</td>
</tr>
<tr>
<td>HBsAg, log IU/mL</td>
<td>2.7 (2.0–3.0)</td>
</tr>
<tr>
<td>HBcrAg, log U/mL</td>
<td>3.0 (2.0–3.9)</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>27 (23–35)</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>27 (19–40)</td>
</tr>
<tr>
<td>Anti-viral regimen</td>
<td></td>
</tr>
<tr>
<td>Entecavir</td>
<td>123 (60.6)</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>80 (39.4)</td>
</tr>
<tr>
<td>Duration on therapy, months</td>
<td>36.9 (36.5–40.1)</td>
</tr>
<tr>
<td>Duration of follow-up, months</td>
<td>31.7 (16.7–67.1)</td>
</tr>
<tr>
<td>Pre-treatment positive HBeAg</td>
<td>32 (15.8)</td>
</tr>
<tr>
<td>Pre-treatment positive anti-HBe</td>
<td>168 (82.8)</td>
</tr>
<tr>
<td>Pre-treatment AST, U/L</td>
<td>68 (40–123)</td>
</tr>
<tr>
<td>Pre-treatment ALT, U/L</td>
<td>103 (54–212)</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase; Anti–HBe, hepatitis B e antibody; AST, aspartate transaminase; HBcrAg, hepatitis B core–related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen. *Status at the cessation of anti–viral therapy unless otherwise specified; values expressed as number (%) or median (interquartile range).

**Figure 1.** The cumulative incidence of clinical relapse following discontinuation of nucleos(t)ide analogues in the study population.

**Figure 2.** The cumulative incidence of clinical relapse according to serum level of hepatitis B core–related antigen measured at the end of treatment. EOT, end-of-treatment; HBcrAg, hepatitis B core–related antigen.
points were illustrated in Supplementary Figures 2 and 3, respectively.

We further clarified the pattern of posttreatment changes in HBcrAg and explored the association with CR. Among the 114 patients who did not resume antiviral therapy at year one, serum HBcrAg decreased in 39 patients (34.2%), did not change in 62 patients (54.4%) and increased in 13 patients (11.4%). The incidence of subsequent CR did not differ between patients with and without decrease in HBcrAg in the first year ($P=0.74$; Fig. 5A). During the second year, serum HBcrAg decreased in 16 (22.5%), did not change in 41 (57.8%), and increased in 14 patients (19.7%). Similarly, there were no difference in the risk of CR between patients with and without HBcrAg decreases in year two ($P=0.84$; Fig. 5B).

**Time-dependent Cox models to examine posttreatment HBcrAg level as an independent risk factor for CR**

In the univariable analysis, male sex, EOT HBsAg, time-varying HBsAg, EOT HBcrAg, time-varying HBcrAg, time-varying HBV DNA, and EOT ALT level were linked to a higher risk of CR, while longer therapy duration was associated with a lower risk (Table 2). In the multivariable model, the post-treatment time-varying HBcrAg level remained a significant predictor of CR, with an adjusted hazard ratio (aHR) of 1.53 per log U/mL (95% CI: 1.12–2.08), after adjusting for EOT HBsAg, time-varying HBsAg, and EOT HBcrAg, alongside factors

![Figure 3. The receiver operating characteristic curve of serum end-of-treatment hepatitis B core-related antigen level to predict clinical relapse off nucleos(t)ide analogues. EOT, end-of-treatment; HBcrAg, hepatitis B core-related antigen.](image)

**Table 2. Univariable and multivariable time-dependent Cox proportional hazard models for the risk of clinical relapse**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.00</td>
<td>0.98–1.02</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.16</td>
<td>1.25–3.73</td>
</tr>
<tr>
<td>EOT positive anti-HBe</td>
<td>0.71</td>
<td>0.35–1.46</td>
</tr>
<tr>
<td>EOT HBsAg level, log IU/mL</td>
<td>1.84</td>
<td>1.47–2.30</td>
</tr>
<tr>
<td>Time-varying HBsAg level, log IU/mL</td>
<td>1.71</td>
<td>1.19–2.45</td>
</tr>
<tr>
<td>EOT HBcrAg, log U/mL</td>
<td>1.30</td>
<td>1.10–1.53</td>
</tr>
<tr>
<td>Time-varying HBcrAg level, log U/mL</td>
<td>1.36</td>
<td>1.14–1.63</td>
</tr>
<tr>
<td>Time-varying HBV DNA level, log IU/mL</td>
<td>1.36</td>
<td>1.15–1.61</td>
</tr>
<tr>
<td>EOT ALT, U/L</td>
<td>1.00</td>
<td>1.00–1.01</td>
</tr>
<tr>
<td>Tenofovir use (vs. entecavir)</td>
<td>0.80</td>
<td>0.54–1.18</td>
</tr>
<tr>
<td>Duration on therapy, months</td>
<td>0.99</td>
<td>0.97–1.00</td>
</tr>
<tr>
<td>Pre-treatment positive HBeAg</td>
<td>1.29</td>
<td>0.80–2.08</td>
</tr>
<tr>
<td>Pre-treatment positive anti-HBe</td>
<td>0.83</td>
<td>0.52–1.32</td>
</tr>
</tbody>
</table>

The measurements were conducted at the end of treatment if not otherwise specified.

EOT, end of treatment; ALT, Alanine transaminase; Anti–HBe, hepatitis B e antibody; HBcrAg, hepatitis B core–related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HR, hazard ratio; CI, confidence interval.

that were selected according to statistical significance. In addition to time-varying HBcrAg, independent predictors of CR in the fully adjusted model included EOT anti-HBe positivity (aHR: 0.19; 95% CI: 0.10–0.37) and EOT HBsAg level (aHR: 2.47 per log IU/mL; 95% CI: 1.28–4.77).

For practical clinical use, the association between post-treatment HBcrAg levels and CR risk was further evaluated using a dichotomous approach, with 1,000 U/mL as the cutoff. In the time-dependent multivariable Cox model adjusted for anti-HBe positivity and EOT HBsAg (Table 3), posttreatment HBcrAg levels below 1,000 U/mL (as compared to 1,000 U/mL or above) were associated with a significantly lower risk of CR, with an aHR of 0.41 (95% CI: 0.21–0.81).

**DISCUSSION**

In this multicenter cohort study, we found that dynamic HBcrAg levels were more predictive of CR than static EOT HBcrAg and dynamic HBsAg levels in CHB patients discontinuing entecavir or tenofovir, although the posttreatment change in HBcrAg was generally mild and the pattern (i.e., with or without decrease during the preceding year) was not independently associated with CR. We further validated the

---

**Figure 4.** The bar charts illustrates the proportion of serum HBcrAg levels <3 log U/mL, 3–4 log U/mL, >4 log U/mL measured at treatment cessation (A), one year afterwards (B), and two years afterwards (C) in all patients, categorized by the occurrence or absence of clinical relapse. HBcrAg, hepatitis B core–related antigen; CR, clinical relapse.

**Figure 5.** The cumulative incidence of clinical relapse according to changes in serum level of hepatitis B core–related antigen during the first year (A) and second year (B). HBcrAg, hepatitis B core–related antigen.
results were consistent with posttreatment levels of HBcrAg set < or ≥1,000 U/mL, a convenient cutoff that can be easily applied in clinical practice. These findings implicate that dynamic measurement of serum HBcrAg may inform post-treatment monitoring in CHB patients who stop NA therapy. Previous studies have shown that lower HBsAg or HBcrAg level at EOT were associated with lower rates of relapse after discontinuation of NA.17,20 Sonneveld et al.23 reported in a multicenter study with a median follow-up of 295 weeks that a lower EOT HBcrAg level was related to better outcomes after cessation of NA, including sustained virologic response, HBsAg loss, and a lower ALT flare rate. Other studies suggested that a combination of the EOT HBcrAg level with either HBV RNA or HBsAg level at EOT may be an acceptable predictor of off-therapy relapse.19,24,25 In the present study, we also found the incidence of CR was significantly lower among patients with HBcrAg <1,000 U/mL at EOT. Despite the significant association with CR, EOT HBcrAg level alone is not accurate enough, as indicated by its AUC of 0.61 (95% CI, 0.53–0.69) in our study.

The serum levels of HBsAg and HBcrAg may change over time following NA cessation, and it has been unclear whether dynamic measurements may add to the accuracy of prediction for subsequent CR. In our prior study with 140 patients, we reported that the time-varying gradient of serum HBsAg was associated with both clinical and virological relapse.26 In the current study, we established for the first time that dynamic HBcrAg levels are superior predictors of CR compared to static EOT HBcrAg levels and dynamic HBsAg levels (Table 2).

Currently, only serum HBV DNA and ALT levels are recommended in guidelines for post-treatment monitoring.27 However, elevation of serum ALT is actually a marker of liver injury that already occurs and does not serve to forecast. With regards to serum HBV DNA, viremia typically relapses within months and can rapidly fluctuate. Therefore, it is recommended that serum HBV DNA should be frequently measured, such as monthly measurement for the first three months and then bi-monthly or tri-monthly measurements in the first year, barring relapses.27 In contrast, serum HBsAg and HBcrAg levels generally do not undergo sudden changes in most patients and can stratify patients at different risks of relapses that may not be imminent. Thus, serum HBcrAg may serve as a complementary biomarker to serum HBV DNA in the posttreatment monitoring of patients stopping NA therapy. Further research is warranted to elucidate how HBcrAg and HBV DNA may be combined together in clinical practice.

Table 3. Multivariable-adjusted analyses to examine posttreatment HBcrAg as a time-varying dichotomous predictor with the cutoff set at 1,000 U/mL

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOT positive anti-HBe</td>
<td>0.42</td>
<td>0.29–0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EOT HBsAg, log IU/mL</td>
<td>1.24</td>
<td>0.95–1.62</td>
<td>0.120</td>
</tr>
<tr>
<td>HBcrAg &lt;1,000 U/mL (vs. ≥1,000 U/mL)</td>
<td>0.41</td>
<td>0.21–0.81</td>
<td>0.010</td>
</tr>
</tbody>
</table>

EOT, end-of-treatment; Anti-HBe, hepatitis B e antibody; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.; HR, hazard ratio; CI, confidence interval.

*As a time varying variable.
mens differ, the design of this study more closely reflects daily clinical practice. Fourth, the follow-up period was sufficiently long enough to observe the outcomes after cessation of NA. Even though the incidence of hepatitis flares was high, and many patients resumed NA and discontinued observation, more than one-third of the patients (n=74, 36.5%) in this cohort remained at risk of CR after three years of follow-up. Finally, our multivariable Cox proportional hazard analysis was developed with the adjustment for EOT HBsAg level and EOT HBcrAg level that were important factors associated with subsequent CR after NA cessation.

There are several limitations to this study. First, due to the health insurance policy in Taiwan, the present study enrolled patients with a median treatment duration of 36.9 months (IQR, 36.5–40.1). The small range of the treatment duration may lead to selection bias, and we could not examine the association between the duration and outcome of treatment. Second, we could not precisely quantify serum HBcrAg levels when HBcrAg levels were lower than 1,000 U/mL. However, the same commercial assay was employed in several prior studies, and is widely used in the clinic. Third, this cohort only included Asian patients and the most common HBV genotypes were type B or C. Finally, we could not exactly investigate the association between the relapse risks and the different patterns of patients with non-decreased HBcrAg levels because of the limited number of patients who remained untreated. Therefore, future studies of more participants with different ethnicities, HBV genotypes, and NA treatment durations are required to validate our findings.

In conclusion, this study revealed that the dynamic measurements of serum HBcrAg after cessation of NA outperformed the EOT HBcrAg level and was more accurate than the dynamic HBsAg level, as an independent predictor for subsequent CR. HBcrAg level <1,000 U/mL could be a useful cutoff value to forecast a low risk of subsequent CR during the off-therapy follow-up. These finding may help to design a safer monitoring strategy for patients who discontinue NA and may inspire further research to optimize the finite strategy of NA therapy.

Authors’ contribution

Acknowledgements
The authors gratefully to our colleagues who treated the study participants. This work was funded by Cha Da Hospital (EDCHP111007), the National Science and Technology Council in Taiwan (110-2314-B-214-006-MY3), and the Tomorrow Medical Foundation (112-1). The authors declare no conflict of interest relevant to the study.

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

28. Su TH, Yang HC, Tseng TC, Liou JM, Liu CH, Chen CL, et al. Distinct relapse rates and risk predictors after discontinuing teno-


Letter regarding “Treated chronic hepatitis B is a good prognostic factor of diffuse large B-cell lymphoma”

Chi Hsiao¹ and Yung-Po Liaw²,³
¹School of Medicine, Chung Shan Medical University; ²Department of Public Health and Institute of Public Health, Chung Shan Medical University; ³Department of Medical Imaging, Chung Shan Medical University, Taichung, Taiwan

Keywords: HBV; Non-Hodgkin lymphoma; Antiviral agent

Dear Editor,

We read with great interest the recent retrospective cohort study by Park et al., entitled “Treated chronic hepatitis B is a good prognostic factor of diffuse large B-cell lymphoma.” In the study, the authors compared the prognostic outcomes of two diffuse large B-cell lymphoma (DLBCL) groups from Seoul University Hospital and Seoul National University Hospital: patients with chronic hepatitis B virus (HBV) infection who received antiviral treatment and patients without HBV infection who did not receive antiviral treatment. The main finding of the article was that the time to progression, progression-free survival, and overall survival were significantly increased for the HBV-infected patients who received antiviral treatment. Meanwhile, I have some additional thoughts and considerations regarding the article.

First, we would like to compare this study with another by Lemaitre et al. that investigated the characteristics and outcomes of HBV non-Hodgkin lymphoma (HBV-NHL) in HBV non-endemic countries. Lemaitre et al. found that when DLBCL is treated with R-CHOP and antivirals, patients (n=24) with HBV infection have similar outcomes to the non-HBV-infected patients. In addition, the median age of the patients with HBV-NHL was 59 years, which is close to that shown by Park et al. (56 years). These two studies suggest that treating HBV might be beneficial for most patients with DLBCL, whether they are in prevalent or non-prevalent areas.

Second, considering the maximum age of the patients in the study is no more than 71 years, it is necessary to further investigate the effect of antiviral treatment on older adult patients with HBV-associated DLBCL in this aging world. A review by Arcari et al. showed recent advances in treatment options for older adult patients with DLBCL, such as polatumab, vedotin, and tafasitamab, yet additional research is needed regarding the effects of antiviral treatment.

In conclusion, we appreciate the valuable work by Park et al., which provided new insight into the treatment of patients with HBV-associated DLBCL. If it is possible to broaden the scope to include wider age ranges and non-endemic areas, we can gain a more comprehensive understanding of...
the treatment’s efficacy and ensure that it caters to a wider population.

Authors’ contributions

Drafting and writing of the article: Chi Hsiao. Supervision: Yung-Po Liaw.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES


Abbreviations:

DLBCL, diffuse large B-cell lymphoma; HBV, hepatitis B virus; HBV-NHL, HBV non-Hodgkin lymphoma
Letter 1 regarding “Assessing the performance of ChatGPT in answering questions regarding cirrhosis and hepatocellular carcinoma”

Hinpetch Daungsupawong¹ and Viroj Wiwanitkit²,³

¹Private Academic Consultant, Phonhong, Lao People’s Democratic Republic; ²Research Center, Chandigarh University, Mohali, India; ³Department of Biological Science, Joseph Ayobabalola University, Ikeji-Arakeji, Nigeria

Keywords: ChatGPT; Cirrhosis; Hepatocellular carcinoma; Liver; AI

Dear Editor,

Regarding the study on “Assessing the performance of ChatGPT in answering questions about cirrhosis and hepatocellular carcinoma¹,” the objective was to evaluate ChatGPT’s precision and consistency in providing information, offering management advice, and delivering emotional support to patients with cirrhosis and hepatocellular carcinoma (HCC). The researchers graded ChatGPT’s responses to 164 questions, evaluated its effectiveness using questionnaires and quality metrics, and assessed its capability for providing emotional support.

The study’s findings revealed that ChatGPT had a thorough understanding of cirrhosis (79.1% correct) and HCC (74.0% correct); however, only a small percentage of its responses were considered comprehensive. Compared to diagnosis and preventive medicine, it performed better in the areas of fundamental knowledge, lifestyle, and treatment. ChatGPT successfully responded to 76.9% of the quality measure queries; however, it failed to provide precise decision-making cutoffs and durations of treatment. Additionally, the model lacks understanding of regional variations in guidelines, such as HCC screening standards. In terms of subsequent steps and adapting to a new diagnosis, it did offer patients and caregivers useful guidance and support.

This study’s weakness is that it only assessed ChatGPT’s effectiveness in giving help and information regarding cirrhosis and HCC. Therefore, the generalizability of the study’s findings to other medical conditions or specialties is limited. The study also did not examine the potential biases or limits of ChatGPT’s answers, such as the reliability of the information it provides or the source of its training data.

Corresponding author: Hinpetch Daungsupawong
Private Academic Consultant, Phonhong, 10000 Lao People’s Democratic Republic
E-mail: hinpetchdaung@gmail.com
https://orcid.org/0009-0002-5881-2709

Viroj Wiwanitkit
Research Center, Chandigarh University, Mohali, Punjab, 140413 India
Tel: +91 1800 121 288 800, E-mail: vwiroj@yahoo.com
https://orcid.org/0000-0003-1039-3728

Editor: Ji Won Han, Catholic University of Korea, Korea
Received: Oct. 5, 2023 / Revised: Oct. 9, 2023 / Accepted: Oct. 11, 2023

Copyright © 2024 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.
Further research is needed to assess ChatGPT’s effectiveness across different medical specialties and conditions and to address the identified limitations. For the model to be successfully integrated into patient care, it is essential to assess how well it can comprehend and interpret complicated medical information, fill in knowledge gaps, and guarantee the correctness and dependability of its responses.

Advanced algorithms and substantial training sets are essential to minimize biases and errors in chatbots. This is because relying solely on one major data source may lead to issues. Chatbot use presents ethical questions due to the potential for unexpected or undesirable outcomes. To stop the spread of false information and harmful ideas, ethical standards and restrictions must be put in place as artificial intelligence language models continue to develop.

Authors’ contributions
Hinpech Daungsupawong 50% ideas, writing, analyzing, approval. Viroj Wiwanitkit 50% ideas, supervision, approval.

Conflicts of Interest
The authors declare no conflicts of interest.

REFERENCES

Abbreviations:
HCC, hepatocellular carcinoma
Dear Editor,

We read with great interest the recently published research analyzing the performances of ChatGPT with respect to the management of cirrhosis and hepatocellular carcinoma. In addition to these advanced liver diseases, steatotic liver disease (SLD) also represents a considerable burden on global health, as it affects one-third of the worldwide population. SLD requires long-term self-management and continuous support. This stems from its slow progression, the emphasis on lifestyle changes, and the constant need for regular patient-physician interactions. Therefore, for patients diag-

**Keywords:** Natural language processing; Artificial intelligence; Non-alcoholic fatty liver disease; Patient education as topic
nosed with SLD, education plays a pivotal role in understanding, managing, and possibly reversing their condition. In our evolving digital era, large language models (LLMs), which are sophisticated generative AI systems trained on vast volumes of data that are capable of producing human-like textual responses, have emerged as promising aids for patient education, particularly in facilitating interactions through natural language dialogues. However, given that the efficacy of LLMs in advancing SLD patient education might vary, it is imperative to compare their performances. Therefore, we conducted a comparative evaluation study to assess the performance of five leading LLMs in responding to SLD-related queries.

Our study was performed between Sep 8th and 28th, 2023. We curated 30 common SLD-related queries spanning domains such as risk factors, clinical test and diagnosis, treatment, follow-up, and prognosis based on guideline-based topics and our clinical experience (Table 1). As a separate and independent prompt, each query was posed to five LLMs: ChatGPT-3.5, ChatGPT-4, Google Bard, Meta Llama2 and Anthropic Claude2, which yielded a total of 30 responses per LLM-chatbot. The generated responses were then randomly ordered within each set of questions and stripped of revealing information (e.g., statements such as “I'm not a doctor” from ChatGPT) to blind reviewers to the LLM-specific response identity. Three seasoned attending-level physicians independently graded the responses as either “appropriate” or “inappropriate” over five rounds, each on a separate day, with an overnight washout interval in between to mitigate memory bias (Supplementary Fig. 1). Specifically, the responses were graded as “appropriate” when they were free from errors and “inappropriate” when they contained potential factual errors that could harm or mislead the average patient. The final grade for each chatbot response was determined using a majority consensus approach, based on the grade most often assigned by the three expert graders.

We assessed the performances of the five LLMs in responding to SLD-related queries. As shown in Table 1, ChatGPT-4 provided 29 of 30 (96.7%) appropriate responses, followed by Bard and Llama2 with 27 of 30 (90.0%), and ChatGPT-3.5 and Claude2 both with 24 of 30 (80.0%), Chi-square test $\chi^2=6.17, P=0.18$. A notable area of concern was the frequent oversimplification of fatty liver disease as synonymous with nonalcoholic fatty liver disease (NAFLD). This oversimplification can lead to inaccuracies. For example, ChatGPT-3.5 replied to the question “Are there different stages of fatty liver disease, and how do they differ?” with the following response: “Yes, there are different stages of fatty liver disease, which is also known as nonalcoholic fatty liver disease (NAFLD). ... The stages of NAFLD are typically categorized as follows: 1. Simple Steatosis (Fatty Liver); 2. Nonalcoholic Steatohepatitis (NASH); ... 3. Fibrosis; 4. Cirrhosis; ...”

This rigorous evaluation study revealed that, among five state-of-the-art LLMs, ChatGPT-4 could generate largely appropriate responses to patient queries regarding SLD, boasting an impressive appropriateness rate of 96.7%. Other LLMs provided 80% to 90% appropriate responses. Health literacy—commonly defined as the degree to which individuals have the skills and abilities to obtain, process, and utilize health-related information—has emerged as a critical priority in reducing inequities among patients, including those with SLD. Our findings underscore the varied potential of LLM chatbots to provide professional yet patient-friendly health literacy guidance to SLD patients. Whereas prior investigations predominantly focused on ChatGPT3.5, our study offers a comprehensive assessment of popular LLMs, namely ChatGPT-3.5, ChatGPT-4, Bard, Llama2 and Claude2, and we specifically evaluated their proficiency in addressing typical SLD-related patient queries. Notably, one in five responses from ChatGPT-3.5 and Claude2 was inappropriate, thus highlighting the need for further iterations and probably domain-specific fine-tuning. Although the exact parameters of ChatGPT-4 remain undisclosed, its impressive performance may result from the large parameter set, extensive user feedback, advanced reasoning abilities, and the integration of insights from previous models into the system. This study derived benefits from implementing a robust study design with proper randomization, wash-out periods and a majority consensus grading process. However, there are also limitations. These sample queries may represent only a small proportion of real-world scenarios. In addition, as the field of LLM evolves at an unprecedented speed, future research is

**Abbreviations:**
- SLD, steatotic liver disease
- LLMs, large language models
- NAFLD, nonalcoholic fatty liver disease
- MASLD, metabolic dysfunction associated steatotic liver disease

## Table 1. Performance of large language models in addressing patient queries regarding steatotic liver disease

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>GPT-3.5</th>
<th>GPT-4</th>
<th>Bard</th>
<th>Llama2</th>
<th>Claude2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Who is more likely to get fatty liver disease?</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>What type of diet can help better manage fatty liver disease?</td>
<td>1</td>
<td>0*</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>How does alcohol consumption affect my fatty liver disease, and should I abstain from alcohol completely?</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>What type and amount of physical activity is recommended for someone with fatty liver disease?</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>I have a lean build; how did I develop fatty liver disease?</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>How does my family’s health history impact the monitoring of my fatty liver disease?</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test and diagnosis</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>What are the early signs and symptoms of fatty liver disease that I should be aware of?</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>How is fatty liver disease diagnosed?</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Are there different types of fatty liver disease, and how do they differ?</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Are there different stages of fatty liver disease, and how do they differ?</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>At what point is a liver biopsy recommended for individuals with fatty liver disease?</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>What is the role of imaging tests such as ultrasound, MRI, or CT scan in diagnosing fatty liver disease?</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>I have fatty liver disease and my ALT is 100 U/L; how should I interpret this?</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>I have fatty liver disease and my FIB-4 score is 1.1; how should I interpret this?</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>How is fatty liver disease treated?</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Are there any specific medications that are commonly prescribed for fatty liver disease?</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>How should medication be used to avoid liver damage in fatty liver disease?</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>What lifestyle interventions can aid in the treatment of fatty liver disease?</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>In severe cases, are there surgical options available for treating fatty liver disease?</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow up and monitoring</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>How often should I be monitored if I have fatty liver disease?</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>I have fatty liver disease. What tests or procedures will be performed during follow-up appointments?</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>I have fatty liver disease. What signs or symptoms should prompt me to seek immediate medical attention?</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comorbidities and prognosis</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>What other health conditions are commonly associated with fatty liver disease?</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>What is the typical prognosis for someone with fatty liver disease?</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
needed to confirm whether LLMs are adapting to new nomenclatures, such as metabolic dysfunction associated steatotic liver disease (MASLD). Generative AI with LLMs—especially ChatGPT-4—may offer yet further valuable insights into opportunities for patient education about SLDs.

Authors’ contributions
Acquisition of data: Yiwen Zhang, Hongwei Ji, Liwei Wu, Zepeng Mu. Analysis and interpretation of data: Hongwei Ji. Drafting of the manuscript: All authors. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Yiwen Zhang Hongwei Ji. Obtained funding: Hongwei Ji. Study supervision: Hongwei Ji.

Acknowledgements
This study was funded in part by the National Key R & D Program of China (2022YFC2502800), National Natural Science Foundation of China (82103908), the Shandong Provincial Natural Science Foundation (ZR2021QH014), the Shuimu Scholar Program of Tsinghua University, and National Postdoctoral Innovative Talent Support Program (BX20230189). The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflicts of Interest
The authors declare no conflicts of interest.

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

REFERENCES


6. 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.

7. Carroll AM, Rotman Y. Nutrition literacy is not sufficient to induce needed dietary changes in nonalcoholic fatty liver disease. Am J Gastroenterol 2023;118:1381-1387.


Letter regarding “Waiting for the changes after the adoption of steatotic liver disease”

Kuo Chao Yew¹, Sunny H. Wong¹,², Vincent Wai-Sun Wong³,⁴, and Hazel H. Oon⁵

¹Department of Gastroenterology and Hepatology, Tan Tock Seng Hospital, Singapore; ²Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; ³Medical Data Analytics Centre, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong; ⁴State Key Laboratory of Digestive Disease, Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong; ⁵National Skin Centre and Skin Research Institute of Singapore (SRIS), Singapore

Keywords: Fatty liver; Metabolic; Alcohol; NAFLD; Social policies

Dear Editor,

We read with interest the recent review by Yoon and Jun.¹ We further describe the challenges and limitations in the implementation of this shift to the new classification of steatotic liver disease (SLD)² in Asia.

SLD is the new umbrella term covering Metabolic Dysfunction-Associated SLD (MASLD), MetALD (MASLD and increased alcohol intake), alcohol-associated liver disease (ALD), specific aetiology and cryptogenic SLD,² and has been met with much debate.

However, discriminating between metabolic and alcohol-associated hepatic steatotic disorder is complex. The assessment of problematic alcohol use remains challenging due to limitations in alcohol intake assessment, lack of non-invasive diagnostic methods for alcohol-associated hepatitis,³ and the unmet need to unify the definition of Metabolic Syndrome (MetS). There is notable discrepancy in the prevalence of MetS across different countries or territories, such as the United Kingdom (34.2%), Japan (25.2%), Taiwan (22.5%), and Italy (30.1%).⁴ It is plausible that these variations arise in part from differences in diagnostic criteria employed. National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), International Diabetes Foundation, World Health Organization, and European Group for the Study of Insulin Resistance, have put forth their own criteria for diagnosing MetS. Of particular relevance to Asia is the modified NCEP-ATP III guidelines, which propose lower waist circumference cut-off thresholds compared to standard NCEP-ATP III guidelines. In a Singapore study by Chan et al.⁴ on the prevalence of MetS among psoriasis patients, utilizing the original criteria yielded a MetS prevalence of 33%, while application of the modified criteria raised this figure to 45.1%. This divergence in prevalence underscores the critical importance of consistent and standardized diagnostic criteria for MetS across different populations, as this significantly alters the epidemiological landscape of MetS.

This is in contradistinction to the high concordance rates of MASLD, non-alcoholic fatty liver disease (NAFLD) and metabolic-associated fatty liver disease (MAFLD) diagnoses.

Corresponding author : Kuo Chao Yew
Department of Gastroenterology and Hepatology, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433
Tel: +65 63577897, Fax: +65 63573087, E-mail: kcyewttsh@gmail.com
https://orcid.org/0000-0003-2005-675X
In a Hong Kong study of 277 participants with intrahepatic triglyceride content ≥5% on proton-magnetic resonance spectroscopy, 89.2% fulfilled criteria for all three definitions.\(^5\) Among the NAFLD cases, only 2.3% and 5.4% failed to meet metabolic criteria of MASLD and MAFLD, respectively.

Recent studies on the genetic aetiology have revealed considerable overlap between MASLD and ALD. Specifically, variants in the genes PNPLA3, TM6SF2, and MBOAT7 have been strongly associated with an increased risk for both conditions.\(^6\) The PNPLA3 I148M variant is the most widely validated genetic determinant and linked to severity of alcoholic cirrhosis in ALD. TM6SF2 and MBOAT7 variants have been reported to confer a risk for progressive disease in both metabolic dysfunction-associated steatohepatitis (MASH) and ALD. Furthermore, it has been shown that MASLD can be driven by endogenous production of ethanol derived from the microbiome.\(^7,8\) These studies indicate the presence of shared biological pathways driving both metabolic and alcohol-related liver injury. These substantial overlaps challenge the binary framework often used to distinguish between metabolic and alcohol-related liver diseases.

The assimilation of the new terminology for SLD demands strategic interventions. Standardized alcohol consumption assessment, harmonized electronic medical records, integration of artificial intelligence,\(^9\) and automatic flagging for MetS, represent significant avenues for clinical management. Employing a standardized radiological report, complemented by cues to categorise underlying risk factors for MASLD/MetALD/MASH, replacing “fatty” with more neutral terms like “lipid/cholesterol/oil,” underscores the relevance of metabolic disorders and alcohol usage.

Adopting a pathogenesis-based approach is essential. This offers deeper insights into SLD’s complexity, guiding tailored management strategies aligned with underlying causes. SLD nomenclature should also encompass other liver aetiologies.\(^10\)

Social and nutrition prescribing have been recently lauded as promising avenues to address the social, economic and mental needs of obesity in MASLD.\(^11\) Healthcare professionals refer to non-medical support systems rooted in the community (e.g., support groups, community gardening, music/culinary classes) with attention to the social determinants of health unique to the individual. There is much work for this to be contextualised for the Asian palate, for SLD programmes and community efforts to be streamlined, and for link workers (social prescribing coordinators) to gain familiarity and expertise in SLD case management. Healthy nutrition habits need to be ingrained systematically in early childhood education curriculum and national policies implemented such as front-of-pack labelling, workplace policies for employees with medically significant and risky SLD. Collectively, these strategies endeavour to streamline diagnostics, enrich medical comprehension, nurture heightened patient engagement, reduce clinical load of SLD at the specialist level and pave the way for prevention of liver diseases and success.

In conclusion, the recent progress in redefining the nomenclature and classification of SLD represents a significant advancement in our understanding of this prevalent and enigmatic conditions. The proposed shift towards a more affirmative and comprehensive approach has the potential to transform clinical practice and research paradigms in management of liver diseases. However, for Asia, the existing challenges in differentiation and accurate classification of SLD underscore the need for ongoing interdisciplinary collaboration, practical implementation and innovative diagnostic tools.

Authors’ contributions

Conception or design of the work: Kuo Chao Yew, Hazel H. Oon. Drafting the article: Kuo Chao Yew, Sunny H. Wong, Hazel H. Oon. Critical revision of the article: All authors. Final approval of the version to be published: All authors.

Abbreviations:

SLD, steatotic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MetALD, metabolic dysfunction-associated steatotic liver disease and increased alcohol intake; ALD, alcohol-associated liver disease; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis
Conflicts of Interest

Kuo Chao Yew: speaker for Gilead Sciences and AbbVie, clinical investigator for AstraZeneca.
Sunny H. Wong: Advisory committee member for Groken Biosciences, Biocodex, and Aptorum Group Limited; speaker for Janssen and AstraZeneca.
Vincent W. Wong: advisory committee member for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Intercept, Inventiva, Merck, Novo Nordisk, Pfizer, Sagimet Biosciences and TARGET Pharma Solutions; speaker for Abbott, AbbVie, Gilead Sciences, Novo Nordisk and Unilab. Received a research grant from Gilead Sciences and is a cofounder of Illuminatio Medical Technology.
Hazel H. Oon: speaker, advisory board member and researcher for AbbVie, Galderma, Janssen and Novartis. Clinical investigator for Pfizer, advisory board member for Amgen, speaker and advisory board member for Boehringer Ingelheim and Eli Lilly.

REFERENCES

10. George J. Adding to the confusion in more than just the name. Clin Mol Hepatol 2023;29:973-976.
Changing from NAFLD to MASLD: Similar cumulative incidence of reflux esophagitis between NAFLD and MASLD

Shuhei Fukunaga, Michita Mukasa, Dan Nakano, Tsubasa Tsutsumi, and Takumi Kawaguchi
Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

Keywords: Steatotic liver disease; Metabolic; Steatosis; Gastroesophageal reflux disease

Dear Editor,

We read the multi-society statement regarding the updated fatty liver disease nomenclature published by the non-alcoholic fatty liver disease (NAFLD) Nomenclature Consensus Group with great interest. NAFLD is now termed metabolic dysfunction-associated steatotic liver disease (MASLD).

Reflux esophagitis is a common gastrointestinal ailment that affects 10–20% of the general population, and its prevalence continues to increase. Major complications of reflux esophagitis include esophageal stricture, esophageal bleeding, Barrett’s esophagus, and esophageal adenocarcinoma. Moreover, reflux esophagitis is associated with anxiety, depression, and impaired patient-reported outcomes.

Reflux esophagitis is often associated with aging, hiatal hernia, and lifestyle-related factors, such as poor eating and exercise habits, and metabolic dysfunction. Furthermore, a recent study reported that NAFLD increases the risk of reflux esophagitis.

Hagström et al. previously reported that >99.5% of patients with NAFLD meet the MASLD criteria and that there is no statistical difference in the rate or risk of overall mortality or liver-related outcomes. In addition, Yoon and Jun found that MASLD criteria encompass almost the entire previous NAFLD patient population (>95%), allowing seamless continuation of ongoing clinical trials for NAFLD drugs. Furthermore, Kim et al. emphasized the significance of preserving and building upon existing NAFLD research to avoid unnecessary waste of research resources. However, no studies have examined the association between NAFLD and MASLD during the development of reflux esophagitis.

We aimed to compare the incidence of reflux esophagitis between patients with NAFLD and those with MASLD. A total of 18,958 consecutive patients who underwent routine health checks were evaluated using esophagogastroduodenoscopy and abdominal ultrasonography at three institutions in Japan between May 2008 and January 2021. All patients were of Asian origin. Patients who were diagnosed with reflux esophagitis using esophagogastroduodenoscopy at their initial health checkup (n=1,825), lacked data regarding alcohol consumption (n=779), reported the use of proton pump inhibitors at their initial health checkup (n=423), or

Corresponding author: Shuhei Fukunaga
Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
Tel: +81-942-31-7561, Fax: +81-942-34-2623, E-mail: fukunaga_shyuhei@med.kurume-u.ac.jp
https://orcid.org/0000-0002-2715-7564

Editor: Young Chang, Soonchunhyang University Seoul Hospital, Korea
Received: Oct. 27, 2023 / Revised: Nov. 29, 2023 / Accepted: Dec. 1, 2023
had no follow-up esophagogastroduodenoscopy (n=8,315) were excluded. The data of 7,616 patients (58% male; median age 49 years [interquartile range, IQR: 41–57 years], median body mass index: 22.6 kg/m$^2$ [IQR: 20.5–24.8 kg/m$^2$]) were analyzed.

The diagnosis of steatotic liver disease (SLD) was based on the presence of moderate or severe hepatic steatosis on ultrasound. Esophagogastroduodenoscopy was performed after overnight fasting by endoscopists with experience performing >1,000 procedures. GIF-H260, GIF-H260Z, GIF-H290, GIF-H290Z, and GIF-XP290N endoscopes were used (Olympus, Tokyo, Japan). The endoscopy findings followed the modified version of the Los Angeles classification to describe the different grades of esophagitis severity (including grades M and A–D) based on the extent of the esophageal lesions. The development of reflux esophagitis during the follow-up period was the primary outcome.

NAFLD was diagnosed in 1,458 patients, including 214 who did not fulfill the metabolic criteria for MASLD. All 214 individuals were classified as having cryptogenic SLD according to the new nomenclature. As a result, 85.4% of patients with NAFLD were also diagnosed with MASLD. The prevalence of hiatal hernia (18.0% and 18.4%), eating habits (eating within two hours before bedtime at least three times/week; 21.1% and 21.1%, respectively), and exercise habits (at least 30 min/session; 18.0% and 18.4%, respectively) were similar between the NAFLD and MASLD groups. Among the five metabolic risk factors listed in the criteria for MASLD, overweight/obesity or central obesity and low HDL cholesterol were the most and least prevalent risk factors, respectively (Supplementary Table 1).

Differences in the cumulative incidence of reflux esophagitis between the non-SLD and NAFLD/MASLD groups were analyzed using log-rank tests. The cumulative incidence of reflux esophagitis was significantly higher in the NAFLD/MASLD group than in the non-SLD group (Fig. 1). However, the cumulative incidence of reflux esophagitis was not significantly different between the NAFLD and MASLD groups. The five-year cumulative incidence of reflux esophagitis was 22.1% in patients with NAFLD and 22.6% in patients with MASLD. These results indicate that excluding cryptogenic SLD from NAFLD did not alter the cumulative incidence of re-

Abbreviations:
SLD, steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease.
Reflux esophagitis. In addition, there was no significant difference in the risk of reflux esophagitis between patients with NAFLD and those with MASLD.

Therefore, the MASLD diagnostic criteria may be useful for the efficient screening of patients at high risk of developing reflux esophagitis.

Authors’ contribution
Shuhei Fukunaga: study concept, design, and statistical analysis; Michita Mukasa: data extraction, interpretation of data, and critical revision of the manuscript; Dan Nakano: interpretation of data, drafting, and critical revision of the manuscript; Tsubasa Tsutsumi: interpretation of data and critical revision of the manuscript; Takumi Kawaguchi: interpretation of data and critical revision of the manuscript.

Acknowledgements
This research was supported by the Japan Agency for Medical Research and Development (AMED) under grant number JP23fk0210090.

Conflicts of Interest
Takumi Kawaguchi received lecture fees from Janssen Pharmaceutical K.K., Taisho Pharmaceutical Co., Ltd., Kowa Company, Ltd., Otsuka Pharmaceutical Co., Ltd., Eisai Co., Ltd., ASKA Pharmaceutical Co., Ltd., AbbVie GK., and EA Pharma Co., Ltd. The other authors have no conflicts of interest.

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

REFERENCES
Dear Editor,

We thank the LLM-Liver Investigators for commenting on our recent publication. Their commentary presents a comprehensive assessment of the capability of various Large Language Models (LLMs) in providing patient education on liver diseases. The authors’ work to assess various LLMs’ performance in providing patient education on liver diseases is a timely study that contributes to understanding the role of multiple recently-introduced LLMs in healthcare dissemination. In the study, LLM’s high performance in delivering appropriate responses underscores the potential for AI to support healthcare providers in disseminating accurate medical information. By setting a benchmark for LLM performance, the study not only contributes to the academic field but also lays the groundwork for the development of AI-driven patient education tools. Their findings could play a crucial role in bridging the gap in health literacy and ensuring equitable access to medical information across diverse patient populations.

There are several aspects that need clarification for proper interpretation of the results. First, the term “steatotic liver disease” is a recently developed nomenclature. The reliability of LLMs to provide up-to-date information depends on their training with current datasets. As the authors used “fatty liver disease” instead of steatotic liver disease, the study would benefit from disclosure of the end dates of the datasets used to train each LLM to ensure that the information provided meets contemporary standards. Furthermore, using the same terminology as that used in recent guidelines would enhance the study’s applicability and clarity. Second, the methodology behind the question selection process remains unclear in the authors’ study. An explanation of how the 30 questions for each LLM were chosen, potentially from clinical guidelines and the authors’ experience, would solidify the study’s robustness. It would preemptively address con-
cerns regarding the selection of questions that might disproportionately favor the capabilities of LLMs.

Additionally, the “washout” period used in the study to minimize recall bias may raise concerns. Recalling responses from previous rounds may influence subsequent evaluations. Finally, it is unsure if there is any statistically significant difference in the performance among the LLMs as there was no analysis performed.

In conclusion, the authors’ study represents an important contribution to the field of AI in patient education. By addressing the areas outlined above, the study can achieve greater validity and provide a more reliable framework for assessing the capability of LLMs in patient education. It is with great anticipation that the medical community looks forward to additional research that builds on this work. We again congratulate the authors for performing the study to enhance our ability to understand and harness AI’s potential in enhancing patient outcomes and health literacy.

Authors’ contribution
Drafting manuscript: YYH. Critical review and supervision: JDY.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Abbreviations:
LLMs, Large Language Models

Correspondence on Letter regarding “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; ²Hanyang Institute of Bioscience and Biotechnology, Hanyang University, Seoul, Korea

Keywords: Metabolic disease; Fatty liver; Metabolic syndrome; Alcoholic fatty liver

Dear Editor,

We have carefully reviewed the Letter to the editor by Yew et al. and find their discussion on the implementation of the new nomenclature, steatotic liver disease (SLD), to be highly insightful.¹,² Yew et al.² raised crucial issues, with a particular emphasis on the insufficient consideration of differences between Western and Asian countries regarding the new definition of SLD. Additionally, they highlighted the need for extensive discussions in the future regarding whether individuals with alcohol-related liver disease (ALD) should be included in the SLD category or another distinct disease framework. These discussions and research endeavors will undoubtedly contribute to a more comprehensive and refined understanding of liver diseases across diverse populations.

Effect size of cardiometabolic risk factors in Western and Asian patients

It is widely accepted that the impact of individual cardiometabolic risk factors and their respective cut-offs on individuals may differ between Asian and Western populations. First, the new SLD nomenclature applies different cut-offs for the body mass index (BMI) and waist circumference for Western and Eastern populations, other various cardiometabolic risk factors beyond the BMI require Asian-specific cut-offs. Second, the influence of each cardiometabolic risk factor on the progression of SLD and its correlation with adverse cardiovascular outcomes remains unclear. For example, there is a lack of data regarding whether equivalent consideration should be given to SLD individuals with diabetes or those with lower high-density lipoprotein cholesterol. Third, there is a need for additional data to validate whether the definition of metabolic dysfunction, comprising one or more abnormal cardiometabolic risk factors, is clinically reasonable and has a similar impact on Western and Eastern popula-
tions. The scientific rationale for defining metabolic dysfunction as one or more abnormal cardiometabolic risk factors is not entirely clear. Future research is warranted to comprehensively investigate the impact of each cardiometabolic risk factor and its cut-off values on Eastern and Western populations. This validation is crucial for ensuring the clinical relevance and applicability of the defined metabolic dysfunction criteria across diverse populations.

**Binary framework for metabolic liver disease and alcohol-related liver disease**

Metabolic dysfunction-associated steatotic liver disease (MASLD) and ALD share several commonalities in their pathogenesis, including the genetic polymorphisms PNPLA3, TM6SF2, and MBOAT7.³ Accurately determining alcohol intake is a realistic challenge, and it is difficult to clearly distinguish between these two disease entities in the real world. Therefore, adopting a binary framework that strictly separates metabolic liver disease from alcohol-related liver disease may not be entirely realistic or reflective of the underlying pathophysiology in some aspects. Nevertheless, the data on whether the effect of alcohol consumption on MASLD has additive synergistic effects remain inconclusive.⁴ This underscores the complexity of the interactions between metabolic factors and alcohol intake in the development and progression of liver diseases, necessitating further research to elucidate the nuanced relationships between these factors.⁵,⁶

**Adopting a pathogenesis-based approach**

The new SLD nomenclature represents a crucial advance that has the potential to enhance disease awareness and facilitate increased access to appropriate treatments. By aligning with the pathophysiology of the disease, this classification system promotes accurate identification and facilitates timely linkage to care within primary care settings.⁸ The subdivision of SLD into distinct subgroups based on factors such as hepatitis C and alcohol consumption is a judicious approach that acknowledges the diverse etiological factors contributing to fatty liver.

In summary, the new SLD classification, built on a pathogenesis-based model, is well-suited to replace the existing nomenclature of non-alcoholic fatty liver disease. It not only eliminates stigmatization associated with the disease name but also fosters heightened awareness and encourages better linkage to care.⁹ Nevertheless, challenges persist in understanding MASLD. More in-depth research is warranted to explore regional and racial variations related to the extent and impact of cardiometabolic risk factors on the occurrence of liver and cardiovascular diseases. Additionally, investigating whether alcohol consumption has an additive or synergistic effect on MASLD requires further exploration. Addressing these unresolved issues will contribute to a more comprehensive understanding of MASLD and guide effective management strategies.

**Authors’ contribution**


**Acknowledgements**

This research was supported by a grant from the 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**


**Abbreviations:**

SLD, steatotic liver disease; ALD, alcohol-related liver disease; BMI, body mass index; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, nomenclature of non-alcoholic fatty liver disease

9. 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.
Recent updates on pharmacologic therapy in non-alcoholic fatty liver disease

Young Chang, Soung Won Jeong, and Jae Young Jang

Institute for Digestive Research, Digestive Disease Center, Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul, Korea

Keywords: Non-alcoholic fatty liver disease; Pharmacologic therapy; Clinical trial
Since there are currently no US Food and Drug Administration (FDA)-approved drugs for the treatment of non-alcoholic steatohepatitis (NASH), various NASH treatments are under development for a wide range of targets. Several promising agents have recently failed in phase 3 trials, including selonsertib,1 elafibranor, and cenicriviroc,2 and now five pharmacologic agents—obeticholic acid (OCA), resmetirom, aramchol, lanifibranor, and semaglutide—are being evaluated in large, histology-based phase 3 trials (Table 1).

OCA, a farnesoid X receptor agonist, has been demonstrated to reduce hepatic fibrosis without worsening NASH in the 18-month interim analysis of the REGENERATE phase 3 trial,3 and its anti-fibrotic effect and long-term favorable safety profile have recently been validated.4 However, in May 2023, the FDA rejected the new drug application of OCA for precirrhotic NASH, concerning safety issues such as hepatotoxicity, cholelithiasis, and pruritus, while its efficacy is modest. Resmetirom is a liver-targeted, thyroid hormone receptor β (THR-β)-selective agonist. Based on the promising results in the phase 2 clinical trial,5 phase 3 trials evaluating the efficacy of resmetirom in patients with NASH presenting with compensated cirrhosis (MAESTRO-NASH-Outcomes trial) and stage 2–3 fibrosis (MAESTRO-NASH) are ongoing. In the interim analysis of MAESTRO-NASH trial, which included 955 patients, histological NASH resolution and fibrosis reduction endpoints were achieved after 52 weeks of treatment.6 Until now resmetirom is the only drug showing both NASH and fibrosis improvement in a phase 3 trial. The antidiabetic drugs, such as glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and glucose-dependent insulino tropic polypeptide (GIP), are attractive candidates for the treatment of NASH, considering their beneficial metabolic effects. These are incretins that stimulate insulin secretion from pancreatic β cells in response to food ingestion. Semaglutide, a GLP-1 RA, showed a significant dose-dependent NASH resolution without worsening of fibrosis in a phase 2 randomized controlled trial (RCT) involving patients with NASH and stage 1–3 fibrosis.7 A phase 3 trial of semaglutide for NASH-related fibrosis (ESSENCE trial) is currently underway. However, in a recent phase 2 RCT for NASH-related cirrhosis, semaglutide did not improve fibrosis or NASH resolution, although it showed improvements in cardiometabolic parameters.8 Effective drug therapy for NASH-related cirrhosis is challenging, as belafec tin,9 selonsertib,1 and OCA also failed in this field. Tirzepatide, a dual agonist for GIP and GLP-1 receptors recently approved for type 2 diabetes mellitus, has been shown to reduce hepatic fat content as well as weight loss, suggesting a potential benefit for NASH.10,11 Additionally, cotadutide, a dual receptor agonist of GLP-1/glucagon,12 and efocipegtrutide, a novel GLP-1/GIP/glucagon triple-receptor co-agonist,13,14 have shown potential as a treatment for NASH. Since glucagon receptors, unlike GIP and GLP-1, are highly expressed in the liver, glucagon agonism has significant impact on metabolism in the liver and extrahepatic tissues.15 Another antidiabetic drug, dapagliflozin, a sodium-glucose cotransporter-2 inhibitor, has been shown to reduce liver steatosis and fibrosis in small RCTs and meta-analysis.16,17 A phase 3 trial (DEAN trial) to assess the efficacy and safety of dapagliflozin in improving NASH and metabolic risk factors is still ongoing. A phase 3 trial of aramchol (AMOR trial) was initiated on the basis of the observed safety and changes in liver histology in the phase 2b trial.18 After the open-label part of the AMOR trial has met its objective, the manufacturer changed its proposed clinical studies with aramchol meglumine instead of aramchol free acid. Accordingly, initiation of the double-blind part is being suspended upon the manufacturer’s strategy. In a phase 2b clinical trial of lanifibranor in patients with NASH and significant fibrosis, lanifibranor achieved both endpoints of NASH resolution and fibrosis improvement, together with an improvement in lipid profile and insulin resistance.19 A phase 3 trial of lanifibranor in patients with NASH and stage 2–3 fibrosis (NAVIv3 trial) is currently ongoing. FG2F1 is a non-mitogenic hormone that regulates glucose and lipid metabolism and modulates adiponectin secretion. Recently, pegbelfermin has failed to demonstrate significant reductions in liver fibrosis in patients with NASH.20,21 In contrast, pegzoafermin, a long-acting glycopegylated recombinant FG2F1 analogue, led to significant improvements in fibrosis...
Table 1. Clinical trials evaluating the efficacy of various agents for nonalcoholic steatohepatitis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Phase</th>
<th>Trial</th>
<th>Fibrosis stage</th>
<th>Size (n)</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obeticholic acid</td>
<td>FXR agonist</td>
<td>3</td>
<td>REGENERATE</td>
<td>NASH fibrosis (F1-3)</td>
<td>2,480</td>
<td>Active, not recruiting FDA rejected the new drug application of OCA for pre-cirrhotic NASH</td>
</tr>
<tr>
<td>Resmetirom</td>
<td>Selective THR-β agonist</td>
<td>3</td>
<td>MAESTRO-NASH</td>
<td>NASH fibrosis (F2-3)</td>
<td>2,000</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Semaglutide</td>
<td>GLP-1 RA</td>
<td>3</td>
<td>ESSENCE</td>
<td>NASH fibrosis (F2-3)</td>
<td>1,200</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>SGLT2 inhibitor</td>
<td>3</td>
<td>DEAN</td>
<td>NASH</td>
<td>100</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Aramchol</td>
<td>SCD1 inhibitor</td>
<td>3</td>
<td>ARMOR</td>
<td>NASH fibrosis (F2-3)</td>
<td>2,000</td>
<td>Part I (open-label) met its objective Part II (double-blind) was suspended</td>
</tr>
<tr>
<td>Lanifibranor</td>
<td>Pan-PPAR agonist</td>
<td>3</td>
<td>NATIV3</td>
<td>NASH fibrosis (F2-3)</td>
<td>2,000</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Pegozafermin</td>
<td>FGF21 agonist</td>
<td>2</td>
<td>ENLIVEN</td>
<td>NASH fibrosis (F2-3)</td>
<td>222</td>
<td>Completed Fibrosis significantly improved ≥1 stage without worsening of NASH</td>
</tr>
</tbody>
</table>

FDA, US Food and Drug Administration; OCA, obeticholic acid; GIP, glucose-dependent insulinotropic polypeptide; GLP-1 RA, glucagone-like peptide-1 receptor agonists; FGF, fibroblast growth factor; FXR, farnesoid X receptor; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl coenzyme A desaturase; SGLT, sodium-glucose cotransporter; THR, thyroid hormone receptor.
and NASH resolution in patients with stage 2–3 fibrosis (EN-LIVEN trial). These promising results provide the basis for the advancement of pegozafermin into phase 3 development. An update on current pharmacological therapies for non-alcoholic fatty liver disease is summarized in Figure.

Several agents, including THR-ß-selective agonist, antidiabetic drugs, and FGF21 agonists show promise as new therapeutics for NASH, and their efficacy in improving both inflammation and fibrosis, with a long-term safety profile, is expected. Considering the heterogeneity of the disease and the various treatment responses observed in clinical trials for individual drugs, it is challenging to define effective drugs for all patients with NASH. Accordingly, combination treatment or personalized treatment approaches may provide a higher response in the context of disease mechanisms.

Authors’ contribution
Young Chang drafted and revised the manuscript. Soung Won Jeong designed the concept and revised the manuscript. Jae Young Jang conducted the literature search and reviewed the manuscript.

Acknowledgements
This work was supported by the Soonchunhyang University Research Fund and the National Research Foundation of Korea (NRF) grant funded by the Korea government (2021R1I1A3059993, RS-2023-00251607).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Erratum to ‘Correspondence on Letter regarding “Evidence-based hyponatremia management in liver disease”’ [Clin Mol Hepatol 2023;29:1048-1049]

Seon Ha Baek¹, Ji Young Ryu¹, 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

The order of author names is incorrect. The author list should be corrected as follows.

The order of listing “Authors’ contribution” has also been modified.

Corrected Author list

Seon Ha Baek, Ji Young Ryu, and Sejoong Kim

Corrected Authors’ contribution

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

Corresponding author : Sejoong Kim

Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7051, Fax: +82-31-787-4051, E-mail: sejoong@snubh.org
ORCID: https://orcid.org/0000-0002-7238-9962
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 construed 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 abstracts 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.
Snapshots

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 copyright 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.

<table>
<thead>
<tr>
<th>Author (Print)</th>
<th>Affiliation</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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>
You can trust EPCLUSA® to deliver consistent outcomes in a variety of settings.1,5k

Robust regimen with some forgiveness to non-adherence7,8,

1 tablet, once a day, with no food requirement2,4

Relatively few clinically relevant DDIs2,3

Proven cure rates,2 even when studied with minimal monitoring1,h

Pi-free

Pan-genotypic2,a and pan-fibrotic2,4d,k

Acknowledged test and treat option2,6,b

Well-characterized on-treatment monitoring in patients with compensated cirrhosis2

Suitable despite uncertainties in liver disease severity3,5,k,l

EPCLUSA® has relatively few clinically relevant DDIs and no limitations around liver disease severity2,3,c–f

Effective PI-free treatment means prescribing with confidence despite unknowns1–6,a–c

You can trust EPCLUSA® to deliver consistent outcomes in a variety of settings.1,5k

Adverse events:

Headache, fatigue and nausea were the most common AEs associated with EPCLUSA® in clinical trials. Headache, fatigue and nausea (incidence ≥10%), as well as other AEs, were reported at a similar frequency in placebo-treated patients. Cardiac disorders, skin rashes and angioedema have been identified during post approval use of sofosbuvir. EPCLUSA® should not be administered concurrently with other medicinal products containing sofosbuvir.

Abbreviations:

AE = adverse event; CPT = Child-Pugh-Turcotte; DDI = drug–drug interaction; EASL = European Association for the Study of the Liver; GT = genotype; HBV = hepatitis B virus; HCV = hepatitis C virus; IV = intravenous; PI = protease inhibitor; RBV = ribavirin; STR = single-tablet regimen; SVR = sustained virologic response at 12 weeks.

Footnotes:

a Despite unknowns in baseline characteristics of some patients, such as: HCV genotype, fibrosis stage, former/current IV drug use, PPI use at baseline and treatment history. b Hepatitis B virus (HBV) reactivation has been reported in HCV/ HBV coinfected patients who were undergoing or had completed treatment with HCV direct acting antivirals, and who were not receiving HBV antiviral therapy. Test all patients for evidence of current or prior HBV infection by measuring HBsAg and anti-HBc before initiating HCV treatment with this drug. In patients with serologic evidence of HBV infection, monitor for clinical and laboratory signs of hepatitis flares or HBV reactivation during HCV treatment with this drug and during post-treatment follow-up. Initiate appropriate patient management for HBV infection as clinically indicated. c In Korea, Epclusa® is indicated for the treatment of adults and pediatric patients 12 years of age and older or weighing at least 30 kg with chronic hepatitis C virus (HCV) genotype 1, 2, 3, 4, 5, or 6 infection, as monotherapy or in combination with ribavirin. d Unless otherwise clinically indicated, there is no need for baseline resistance or on-treatment monitoring of haematology and clinical chemistry in patients receiving EPCLUSA®. e EPCLUSA® offers an RBV-free STR option for the majority of HCV patients, excluding those with compensated cirrhosis. For further information on restrictions please refer to the prescribing information. RBV is recommended for the treatment of patients with decompensated cirrhosis. f No dosage adjustment of this drug is required for patients with mild, moderate, or severe hepatic impairment (Child-Pugh Class A, B, or C). c–f Clinical and hepatic laboratory monitoring (including direct bilirubin), as clinically indicated, is recommended for patients with decompensated cirrhosis receiving treatment with this drug and ribavirin. g On-treatment monitoring may be required for patients with comorbidities or on certain concomi medications. Please refer to prescribing information for further information. h A phase 3 study conducted at 16 sites in India, 129 adult patients with chronic HCV infection of any genotype initiated 12 weeks of once-daily sofosbuvir-velpatasvir (400-100 mg). Study drug was dispensed monthly, but there were no on-treatment study assessments. The primary efficacy endpoint was rate of sustained virologic response (HCV RNA <15 IU/mL) 12 weeks after treatment (SVR12), which was compared to a pre-specified performance goal of 85%. i In a Phase 2 study in patients with chronic HCV and CPT-C cirrhosis, EPCLUSA®+RBV for 12 weeks led to a 78% SVR12 rate. Treatment was well tolerated, with observed AEs consistent with expectations for a patient population with advanced liver disease.5

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. *95% lower price than Original drug (June 2023)
**Remarkable Response**

The ORR was more than three times higher with lenvatinib versus control group.\(^1\)

Based on the masked IIR according to mRECIST,
about 41% of patients\(^*\) showed \(\geq 30\%\) decrease in tumor size.\(^{1,2}\)

**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 text of the article. (Kudo M, et al. 2018)

<table>
<thead>
<tr>
<th>Treatment</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;0.0001</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>

\(^{1}\) mRECIST, modified Response Evaluation Criteria in Solid Tumors; \(^{2}\) IIR, Independent Imaging review; \(^{3}\) ORR, Objective Response Rate; \(^{4}\) CI, Confidence Interval; \(^{5}\) OR, Odds Ratio; \(^{6}\) CSG, Clinical Study Group (Referenced: Kudo M et al, Lancet, 2018 Mar; 391(10078):1564-1573.2, Lancet, 2019 Jul 27; 394(10196):126-136.)

**Lenvatinib**

*"Lenvatinib" is an investigational compound approved by the Ministry of Food and Drug Safety in Korea.\(^{7}\) This is a clinical trial to evaluate the safety and efficacy of lenvatinib in patients with advanced gastrointestinal stromal tumors (GIST). The trial is designed to compare the outcomes of patients treated with lenvatinib versus those treated with sorafenib. The primary endpoint is PFS (progression-free survival), and the secondary endpoints include OS (overall survival), ORR (objective response rate), and safety.\(^{8}\)

\(^{7}\) Ministry of Food and Drug Safety (MFDS), Republic of Korea.\(^{8}\) Data on file only.
Liver Fibrosis Single Biomarker

M2BPGi (Mac-2 Binding Protein Glycosylation isomer) 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.⁵

**TACE treatment for HCC:** The combination of M2BPGi and up-to-seven criteria could serve as a surrogate marker for predicting CP grade deterioration.⁶

**CHB:** The M2BPGi level can predict HCC development independently.

References


Together for a better healthcare journey

www.sysmex.co.kr
Maviret® INDICATIONS: Treatment of adult and adolescent 12 years and older patients with chronic hepatitis C virus (HCV) genotype 1, 2, 4, 5, or 6. It is also indicated for treatment of HCV mono-infected or HCV/HIV-1 co-infected patients with genotype 1, 2, 3, 4, 5, or 6 infection.

- Transplant patients with genotype 1-infection who are NS5A inhibitor-experienced without prior treatment with an NS3/4A protease inhibitor. Prior decompensation is characterized by an abrupt increase in HBV replication manifesting as an increase in serum HBV DNA levels. In patients with resolved HBV infection, HBV reactivation is characterized by HBsAg reappearance, usually within 5 months after initiating antiviral therapy. Patients with co-existing or prior HBV infection should be periodically monitored with clinical and laboratory tests (e.g., HBsAg, HBV DNA, ALT, bilirubin, etc.) to confirm no hepatitis flare or reactivation during and post-treatment follow up. When HBV reactivation has occurred, consult the physician. Use in diabetic patients: Diabetes may experience improved glucose control, potentially resulting in symptomatic hypoglycemia, particularly within the first 3 months, and their diabetic medication modified when necessary. The physician in charge of the diabetic care of the patient should be informed when direct acting antiviral therapy is initiated. Risk of Hepatitis C Decompensation/Failure in Patients with Advanced Liver Disease: Post-marketing cases of hepatic decompensation/failure, including some with fatal outcomes, have been reported in patients treated with HCV NS3/4A protease inhibitor-containing regimens, including MAVIRET. Because these events were 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 comorbidities have had evidence of advanced liver disease, moderate or severe hepatic impairment (Child-Pugh B or C) prior to initiating treatment with MAVIRET, including some patients reported having compensated cirrhosis with mild liver impairment (Child-Pugh A) at baseline but with a prior decompensation event (e.g., prior history of variceal bleeding, encephalopathy), some 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 being a concurrent medication not recommended for coadministration, or in patients with comorbid factors such as severe uncontrolled medical or surgical complications. Cases typically occurred within the first 4 weeks of treatment (median of 27 days). In patients treated with compensated cirrhosis (Child-Pugh A) at baseline of advanced liver disease such as portal hypertension, perform a baseline laboratory test, as clinically indicated, and monitor for any signs or symptoms of hepatic decompensation such as the presence of ascites, jaundice, hepatic encephalopathy, and variceal hemorrhage. Discontinue MAVIRET in patients who develop evidence of hepatic decompensation/failure. MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

**4 WEEKS SOONER OF MAVIRET®**

For GT 1–6 treatment-naïve, non-cirrhotic and compensated-cirrhotic patients, 8-week MAVIRET versus 12-week MAVIRET.

8 WEEKS

MAVIRET is indicated for the treatment of chronic hepatitis C virus (HCV) infection in adults and children aged 12 years and older. Refer to the full Prescribing Information and Summary of Product Characteristics for further details.

**Table 1. Recommended Duration for Treatment-Naive Patients**

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Genotype 1, 2, 4, 5, or 6</th>
<th>1, 2, 4, 5, or 6</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 4, 5, or 6</td>
<td>No Cirrhosis</td>
<td>Compensated Cirrhosis</td>
<td>8 weeks</td>
<td>12 weeks</td>
</tr>
</tbody>
</table>

**Table 2. Recommended Duration for Treatment-Experienced Patients**

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>Genotype 1, 2, 4, 5, or 6</th>
<th>1, 2, 4, 5, or 6</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>An NS3/4A inhibitor without prior treatment with an NS5A inhibitor</td>
<td>12 weeks</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>An NS3/4A inhibitor without prior treatment with an NS5A inhibitor</td>
<td>16 weeks</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**What you should know before taking MAVIRET**

- MAVIRET is contraindicated in patients with mild, moderate, or severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation. Use with caution in patients with mild, moderate, or severe renal impairment, including those patients on dialysis.

- MAVIRET is contraindicated in patients with genetic problems such as galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption.

**Main Warning and Precautions**

1. **Main Warning**

   - Reappearance of HBsAg and HBV reactivation is a rare event. Cases of HBV reactivation, some resulting in liver failure or death, have been reported in patients treated with HCV direct-acting antiviral agents. HBV reactivation is characterized by an abrupt increase in HBV replication manifesting as an increase in serum HBV DNA levels. In patients with resolved HBV infection, HBV reactivation is characterized by HBsAg reappearance, usually within 5 months after initiating antiviral therapy. When HBV reactivation has occurred, consult the physician. Use in diabetic patients: Diabetes may experience improved glucose control, potentially resulting in symptomatic hypoglycemia, particularly within the first 3 months, and their diabetic medication modified when necessary. The physician in charge of the diabetic care of the patient should be informed when direct acting antiviral therapy is initiated.

2. **Contraindication**

   - MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

3. **Cautions**

   - Use with caution in patients with mild, moderate, or severe renal impairment, including those patients on dialysis. Use with caution in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

   - Severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

4. **Drug Interactions**

   - MAVIRET should not be given to patients with genetic problems such as galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption.

5. **Healthcare professional**

   - MAVIRET may cause anaphylaxis, severe and prolonged alteration in blood glucose levels, and serious skin reactions (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis). Because these events were 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 comorbidities have had evidence of advanced liver disease, moderate or severe hepatic impairment (Child-Pugh B or C) prior to initiating treatment with MAVIRET, including some patients reported having compensated cirrhosis with mild liver impairment (Child-Pugh A) at baseline but with a prior decompensation event (e.g., prior history of variceal bleeding, encephalopathy), some 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 being a concurrent medication not recommended for coadministration, or in patients with comorbid factors such as severe uncontrolled medical or surgical complications. Cases typically occurred within the first 4 weeks of treatment (median of 27 days). In patients treated with compensated cirrhosis (Child-Pugh A) at baseline of advanced liver disease such as portal hypertension, perform baseline laboratory test, as clinically indicated, and monitor for any signs or symptoms of hepatic decompensation such as the presence of ascites, jaundice, hepatic encephalopathy, and variceal hemorrhage. Discontinue MAVIRET in patients who develop evidence of hepatic decompensation/failure. MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

**Other warnings**

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.

- MAVIRET is contraindicated in patients with severe hepatic impairment (Child-Pugh B or C) or those with any history of prior hepatic decompensation.

- MAVIRET is contraindicated in patients with severe renal impairment (e.g., end-stage renal disease) or those with any history of prior renal impairment.
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.
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 Antitoxic fraction 12.5mg, Adenine HCl 25mg, Pyridoxine HCl 25mg, Riboflavin 0.3mg, Cyanocobalamin 0.125mg, Biphenyl dimethyl dicarboxylate 25mg
- Indication: 1) 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 3) General caution: 1) Rarely skin rash can be represented, in this case general antihistamin therapy will be required. 2) In severe case, sometimes intermittent jaundice can be occur in this case, discontinue administration for awhile and other adjuvant therapy for jaundice shall be required. 3) Rarely nausea, gastric discomfortness can be represented 4) Rarely itching or redness can be occur, in this case, discontinue administration and follow physician’s instruction
- Insurance Code: 693900000080 1 Packing Unit: 100, 300 caps. (bottle)/ 100 caps. (PFP)
- Storage: Tight closed container, room temperature (1~30°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, Nonalcoholic steatohepatitis  K77.0 Liver disorders in disease classified elsewhere
Gilead Liver Commitment
Exploring for Complete Understanding of Liver Disease
VEMLIDY-for the flow of life with chronic hepatitis B

Vemlidy® for the flow of life with chronic hepatitis B

References

QR 코드를 스캔하여 Vemlidy® PI를 확인하십시오.

---

베믈리디® 정 (테노포바르알레나미드헤미푸마르산염)

[수입자] 길리어드 사이언스 코리아(유), 서울특별시 중구 을지로5길 26 센터원빌딩 서관 15층 (대표전화: 02-6030-3300, 제품관련문의: 0079-814-800-9172 (수신자 부담))
* 처방하시기 전에 반드시 허가사항 전문을 확인하여 주시기 바랍니다. 최신 허가사항은 아래 QR 코드를 통해 확인하실수 있으며, 길리어드사이언스코리아 홈페이지(www.gilead.co.kr) 또는 식품의약품안전처 의약품통합정보 시스템 (http://nedrug.mfds.go.kr) 에서도 확인하실 수 있습니다

KR-VEM-0185_v1.0(29/August/2023)

QR 코드를 스캔하여 Vemlidy® PI를 확인하시십시오.
The new wave of GERD Treatment, P-CAB

FEXUCLUE
Fexuprazan hydrochloride

Excellent nocturnal symptom control: Longest half-life
Significantly improved chronic cough of EE
Take once a day regardless of meal
Less affected by CYP2C19:
Low potential of DDI individual variations

Rapid and superior heartburn symptom relief
Full and fast onset of effect with the first dose
RECOGNIZE & KILL CANCER CELLS
Paradigm Shift in Cancer Treatment

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: Adjuvant 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, every second week, 4 times, once every fourth week, 4 times, every eighth week; 16 times in total.
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!
(Sorafenib tosylate(Micronized))

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

Hanmi Hanmi Pharm.


SOHR-2012-01
Korea’s first COMBIGEL type of Product for a treatment of mixed dyslipidemia

A New Therapy for Mixed dyslipidemia

ATMEG COMBIGEL®
Atorvastatin 5mg, 10mg / Omega-3 1g
SK Albumin

Human serum albumin

- Maintenance of Intravascular pressure
- Acid-base balance
- Drug transport
- Transport of ions, fatty acids, bilirubin and hormones

Summary of Prescribing information

[PRODUCT NAME] SK Albumin 5%/20% [INJECTION]

[CONTENTS] Each 100 mL contains 5 g and 20 g of Human Serum Albumin as active ingredient, for 5% Inj. and 20% Inj., respectively.

[INDICATION AND USAGE] Hypoproteinemia caused by albumin loss (renal, nephrotic syndrome, etc.) and dysfunction of albumin synthesis (liver cirrhosis, etc.), hemorrhagic shock.

[DOSAGE AND ADMINISTRATION] 1. 5% Inj.: 500 mL, equivalent to human serum albumin 25 g should be administered by intravenous drip infusion or by slow direct intravenous injection.

The recommended infusion rate is 2–4 mL/min. The dosage may be adjusted according to body weight, age and symptoms, 2–20% Inj.: 125–375 mL, equivalent to human serum albumin 25–75 g should be administered by intravenous drip infusion or by slow direct intravenous injection. The recommended infusion rate is 2–4 mL/min. It may be diluted with 5% glucose when necessary. The dosage may be adjusted according to body weight, age and symptoms.

[CONTRAINDICATION] Patients with a history of hypersensitivity reactions to the drug and its components.

[MANUFACTURER] SK Plasma Co., Ltd. (39111) 237 Seokodong, Junggu, Andong-si, Gyeongsangbuk-do, Republic of Korea

For the details, you are recommended to check on prescribing information. The latest approved label is available on the website following: http://drug.midx.go.kr

Available from: http://drug.midx.go.kr
Ramnos® not only strengthens intestinal health and immunity, but also improves atopic symptoms.
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(AS, ALT, Pt) 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

에크미메디칼(주) TEL. 02 585 1291
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.

**Tolerance of Besivo**
- Besivo had no drug-resistance mutation for 192 weeks.

**Safety data of Besivo**
- Besivo has a better safety profile than TDF*, in terms of bone and renal outcomes.

**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 660 mg of L-Carnitine together to prevent a decrease in serum L-Carnitine levels. Patients with mild renal impairment: dose adjustment is not required. Patients with moderate renal impairment: it is recommended to administer one tablet once every 2 days for moderate symptoms and one tablet once every 4 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. 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. This drug contains lactose, it should not be administered to patients with genetic problems such as lactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption.
Proven.
Personalized.
Precise.

Your precision strike.
Arming you to target HCC tumors directly and hit them hard with high-dose radiation therapy.
신뢰와 믿음으로
한국인의 위를 지켜온

소화불량증의 1차선택제

가나칸
(Itopride HCl)

NEJM에서 효과와 안전성을 입증받은 소화불량치료제 - ITOPRIDE, 가나칸 3 Yes & 3 No

1st Yes 가나칸은 상복부부터 하복부까지 전체적인 위장관운동을 개선시켜줍니다. 1
2nd Yes 백혈상호작용이 없어 다른 약물과 안전하고 병용이 가능합니다. 2
3rd Yes 가나칸은 일주일이상 정기체크가 가능한 위장관운동치료제입니다. 3

Reference
3. KFDA label
4. Pharma medica 2001;vol19(4)
5. JK Practitioner 2005;
Novo Nordisk at a glance

Novo Nordisk is a leading global healthcare company, founded in 1923 and headquartered in Denmark.

- Products marketed in 170 countries
- Among the world's 10 largest pharma companies measured by market cap
- Supply of nearly 50% of the world's insulin
- Over 30M people use our diabetes care products

Novo Nordisk is dedicated to helping address the unmet needs of people.