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

197 Clinical practice guideline and real-life practice in hepatocellular carcinoma: A Korean perspective
Myung Ji Goh, Dong Hyun Sinn, Jong Man Kim, Min Woo Lee, Dong Ho Hyun, Jeong Il Yu, Jung Yong Hong, and Moon Seok Choi

206 Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Chinese perspective
Diyang Xie, Jieyi Shi, Jian Zhou, Jia Fan, and Qiang Gao

217 Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Hong Kong perspective
Rex Wan-Hin Hui, Lung-Yi Mak, Tan-To Cheung, Victor Ho-Fun Lee, Wai-Kay Seto, and Man-Fung Yuen

230 Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Taiwan perspective
Tung-Hung Su, Chih-Horng Wu, Tsung-Hao Liu, Cheng-Maw Ho, and Chun-Jen Liu

242 Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Japanese perspective
Hironori Koga, Hideki Iwamoto, Hiroyuki Suzuki, Shigeo Shimose, Masahito Nakano, and Takumi Kawaguchi

252 Overview of Asian clinical practice guidelines for the management of hepatocellular carcinoma: An Asian perspective comparison
Yuri Cho, Bo Hyun Kim, and Joong-Won Park

263 The role of different viral biomarkers on the management of chronic hepatitis B
Lung-Yi Mak, Rex Wan-Hin Hui, James Fung, Wai Kay Seto, and Man-Fung Yuen

277 Utility of combining PIVKA-II and AFP in the surveillance and monitoring of hepatocellular carcinoma in the Asia-Pacific region

293 Neuropilins as potential biomarkers in hepatocellular carcinoma: a systematic review of basic and clinical implications
Paula Fernández-Palanca, Tania Payo-Serafin, Carolina Méndez-Blanco, Beatriz San-Miguel, María J. Tuñón, Javier González-Gallego, and José L. Mauriz

320 Chronic hepatitis B with concurrent metabolic dysfunction-associated fatty liver disease: Challenges and perspectives
Shang-Chin Huang and Chun-Jen Liu
Editorials

332 What should be done to reduce the discrepancy between guidelines and real-life practice for hepatocellular carcinoma in Korea?
Min Kyung Park and Yoon Jun Kim

335 Toward user-friendly and evidence-based practice guidelines for hepatocellular carcinoma
Do Young Kim

339 The clinical management of hepatocellular carcinoma in China: Progress and challenges
Shan Shan and Jidong Jia

342 Management of hepatocellular carcinoma in China: Seeking common grounds while reserving differences
Tian Yang, Ming-Da Wang, Xin-Fei Xu, Chao Li, Han Wu, and Feng Shen

345 The prime time for management of hepatocellular carcinoma in Hong Kong
Landon L. Chan and Stephen L. Chan

349 Clinical practice guidelines and real-world practice for hepatocellular carcinoma in Taiwan: Bridging the gap
Shen-Yung Wang

352 Challenges in translating clinical guidelines into real-life practice for management of hepatocellular carcinoma in Taiwan
San-Chi Chen

355 The latest global burden of liver cancer: A past and present threat
Joo Hyun Oh and Dae Won Jun

358 The current trends in the health burden of primary liver cancer across the globe
Peter Kony, Aijaz Ahmed, and Donghee Kim

363 The imitator of immune-tolerant chronic hepatitis B: A killer in disguise
Moon Haeng Hur and Jeong-Hoon Lee

367 Is liver biopsy essential to identifying the immune tolerant phase of chronic hepatitis B?
Joo Hyun Oh and Dong Hyun Sinn

371 Changing the nomenclature from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease is more than a change in terminology
Eileen Laurel Yoon and Dae Won Jun
374 The growing burden of non-alcoholic fatty liver disease on mortality
Ju-Yeon Cho and Won Sohn

377 Lean vs. obese phenotypes of nonalcoholic fatty liver disease: similar or different?
Ho Soo Chun and Minjong Lee

381 Non-obese or lean nonalcoholic fatty liver disease matters, but is it preventable or inevitable in light of its risk factors?
Heejoon Jang and Won Kim

384 Implications of comorbidities in nonalcoholic fatty liver disease
Sherlot Juan Song and Vincent Wai-Sun Wong

390 Screening strategies for non-alcoholic fatty liver disease: a holistic approach is needed
Philipp Kasper, Münevver Demir, and Hans-Michael Steffen

394 Non-invasive imaging biomarkers for liver steatosis in non-alcoholic fatty liver disease: present and future
Lynna Alnimer and Mazen Noureddin

398 Non-invasive biomarkers of liver fibrosis in nonalcoholic fatty liver disease
Maamon Basheer, Mohamed Naffaa, and Nimer Assy

401 Non-invasive biomarkers for liver inflammation in non-alcoholic fatty liver disease: present and future
Kee-Huat Chuah and Wah-Kheong Chan

404 Hepatocellular carcinoma surveillance in non-alcoholic fatty liver disease – who and how?
Margaret LP Teng, Darren Jun Hao Tan, Cheng Han Ng, and Daniel Q. Huang

408 The effect of moderate alcohol consumption on nonalcoholic fatty liver disease
Ji-Won Park and Ki Tae Suk

411 How to optimize the outcome of liver transplantation for non-alcoholic fatty liver disease
Byeong Geun Song and Dong Hyun Sinn

414 The independent effect of exercise on biopsy proven non-alcoholic fatty liver disease: A systematic review
Young-Joo Jin
Original Articles

417 Single-cell phenotypes of peripheral blood immune cells in early and late stages of non-alcoholic fatty liver disease
Kathryn Jane Waller, Hajar Saihi, Wenhai Li, James Hallimond Brindley, Anja De Jong, Wing-kin Syn, Conrad Bessant, and William Alazawi

Sungchul Choi, Beom Kyung Kim, Dong Keon Yon, Seung Won Lee, Han Gyeol Lee, Ho Hyeok Chang, Seoyeon Park, Ai Koyanagi, Louis Jacob, Elena Dragioti, Joaquim Radua, Jae Il Shin, Seung Up Kim, and Lee Smith

453 Factors associated with unrecognized cirrhosis in patients with hepatocellular carcinoma
Yi-Te Lee, Mohammad A. Karim, Hye Chung Kum, Sulki Park, Nicole E. Rich, Mazen Noureddin, Amit G Singal, and Ju Dong Yang

465 Taurocholic acid promotes hepatic stellate cell activation via S1PR2/p38 MAPK/YAP signaling under cholestatic conditions
Jing Yang, Xujiao Tang, Zhu Liang, Mingzhu Chen, and Lixin Sun

482 Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune-tolerant phase
Jeong-Ju Yoo, Soo Young Park, Ji Eun Moon, Yu Rim Lee, Han Ah Lee, Jieun Lee, Young Seok Kim, Yeon Seok Seo, and Sang Gyune Kim

496 Next-generation sequencing analysis of hepatitis C virus resistance–associated substitutions in direct-acting antiviral failure in South Korea
Kyung-Ah Kim, Sejoon Lee, Hye Jung Park, Eun Sun Jang, Youn Jae Lee, Sung Bum Cho, Young Suk Kim, In Hee Kim, Byung Seok Lee, Woo Jin Chung, Sang Hoon Ahn, Seungtaek Kim, and Sook Hyang Jeong

Letter to the Editor

510 Letter regarding “Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune-tolerant phase”
Chia-Ming Chu and Yun-Fan Liaw

Correspondence

513 Correspondence on Letter regarding “Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune tolerant phase”
Jeong-Ju Yoo and Sang Gyune Kim
Snapshot

516 Systemic therapy in advanced hepatocellular carcinoma
Joseph C. Ahn, Nguyen H. Tran, and Ju Dong Yang
Clinical practice guideline and real-life practice in hepatocellular carcinoma: A Korean perspective

Myung Ji Goh1,*, Dong Hyun Sinn1,*, Jong Man Kim2, Min Woo Lee3, Dong Ho Hyun3, Jeong Il Yu4, Jung Yong Hong5, and Moon Seok Choi6

1Division of Gastroenterology and Hepatology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 2Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 3Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 4Department of Radiation Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; 5Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Hepatocellular carcinoma (HCC) is a major cause of death in many countries, including South Korea. To provide useful and sensible advice for clinical management of patients with HCC, the Korean Liver Cancer Association and National Cancer Center Korea Practice Guideline Revision Committee have recently revised the practice guidelines for HCC management. However, there are some differences between practice guidelines and real-life clinical practice. In this review, we describe some key recommendations of the 2022 version of practice guidelines and the real-life clinical situation in South Korea, together with discussion about efforts needed to reduce the difference between guidelines and real-life clinical practice.

(Clin Mol Hepatol 2023;29:197-205)

Keywords: Clinical practice guideline; Hepatocellular carcinoma; Surveillance; Diagnosis; Treatment

INTRODUCTION

In South Korea, liver cancer has the second highest crude death rate and causes the largest economic burden among all types of cancer.1,2 The Korean Liver Cancer Association (KLCA, formerly the Korean Liver Cancer Study Group [KLC- SG]) and National Cancer Center (NCC) of Korea published the first practice guidelines for management of hepatocellular carcinoma (HCC) in 20033 and revised them in 2009, 2014, and 2018.4-6 Since then, new research findings and therapies have accumulated. Accordingly, practice guidelines were revised again in 2022 by integrating the most up-to-date research findings, new therapies, and expert opinions.1 Studies collected for evidence were analyzed through a systematic review, and levels of evidence were classified based on the revised Grading of Recommendations, Assessment, Development, and Evaluation (GRADE).7 In recent years, systemic treatment of HCC has evolved dramatically. Atezolizumab plus bevacizumab has shown superior efficacy over sorafenib and is now considered as a preferred first-line option.1 Sec-
ond-line therapy is urgently needed for patients who have failed treatment with an immune checkpoint inhibitor-based regimen. However, there is little evidence to guide second-line therapy for these patients. Hence, for the first time, a D grade recommendation was described in the KLCA-NCC guidelines.\(^1\) In this review, we summarize the 2022 KLCA-NCC Korea practice guidelines and real-life practice for HCC in South Korea.

**SURVEILLANCE**

**Key recommendations**

The 2022 KLCA-NCC guidelines recommend HCC surveillance in high-risk groups (patients with chronic hepatitis B [A1], chronic hepatitis C [B1], and liver cirrhosis [A1]) with liver ultrasonography (US) plus serum alpha-fetoprotein (AFP) measurement every six months (A1). Guidelines also recommend dynamic contrast-enhanced computed tomography (CT) or dynamic contrast-enhanced magnetic resonance imaging (MRI) as an alternative when liver US cannot be performed adequately (C1).

**Real-life situation and practice**

In South Korea, most of HCC patients are diagnosed at an advanced stage. In an analysis of the Korean Primary Liver Cancer Registry between 2012 and 2014, which was a random sample consisting of 15% of newly diagnosed HCC patients in South Korea, about half were diagnosed at an advanced stage.\(^8\) Timely diagnosis and treatment are suboptimal at the population level.\(^9\) The Korean government initiated the National Liver Cancer Screening Program (NLCSP) in 2003,\(^10\) which offers US and AFP tests for high-risk individuals.\(^11\) According to a nationwide cohort study using the Korean National Health Insurance Service database, only 52.7% of high-risk individuals participated in the NLCSP.\(^12\) To improve adherence to surveillance recommendations in Korea, additional efforts and strategies are needed. Initial presentation of HCC at an advanced stage in patients under regular HCC surveillance is another problem as the surveillance goal is to detect HCC at early stage. However, the sensitivity of US for detecting early-stage HCC is suboptimal,\(^13\) leading to surveillance failure in clinical practice.\(^14,15\) Two Korean prospective studies have evaluated the usefulness of dynamic-contrast CT and MRI with liver-specific contrast for HCC surveillance. Both dynamic-contrast CT and MRI showed higher sensitivity and specificity than US-based surveillance.\(^16,17\) However, alternative imaging methods for HCC surveillance are needed to overcome the limitations of CT and MRI. Hence, the guidelines recommend alternative screening tools only when liver US cannot be performed adequately.

**DIAGNOSIS**

**Key recommendations**

The diagnosis of HCC can be based on pathology or typical hallmarks of HCC obtained by non-invasive imaging for high-risk groups (chronic hepatitis B [A1], chronic hepatitis C [B1], or cirrhosis [A1]). For a new liver nodule ≥1 cm detected by surveillance tests in high-risk patients, multiphasic CT or multiphasic MRI (extracellular contrast agents or hepatocyte-specific contrast agents) should be performed as a first-line imaging study for diagnosis of HCC (A1). If a first-line imaging study is inconclusive for diagnosis of HCC, second-line imaging tests including multiphasic CT, multiphasic MRI, and contrast-enhanced US (blood-pool contrast agents or Kupffer cell-specific contrast agents) can be applied (B1). A diagnosis of “definite” HCC can be based on a nodule ≥1 cm in high-risk patients in the presence of the hallmark arterial phase hyperenhancement with washout appearance (A1 for multiphasic CT or MRI with extracellular contrast agent; B1 for MRI with liver-specific contrast and contrast-enhanced US). A diagnosis of “probable” HCC can be based on ancillary imaging features of HCC (B1). The guidelines include a diag-

**Abbreviations:**

HCC, hepatocellular carcinoma; KLCA-NCC, Korean Liver Cancer Association and National Cancer Center; GRADE, Grading of Recommendations, Assessment, Development, and Evaluation; US, ultrasonography; AFP, alphafetoprotein; CT, computed tomography; MRI, magnetic resonance imaging; NLCSP, National Liver Cancer Screening Program; LI-RADS, Liver Imaging-Reporting and Data System; mUICC, modified Union for International Cancer Control; BCLC, Barcelona Clinic Liver Cancer; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; MWA, microwave ablation; EBRT, external beam radiation therapy; TARE, transarterial radioembolization; ECOG, Eastern Cooperative Oncology Group
Figure 1. Diagnostic algorithm of HCC. HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CT, computed tomography; MRI, magnetic resonance imaging; APHE, arterial phase hyperenhancement; US, ultrasonography. *The radiological hallmarks for diagnosing “definite” HCC on multiphasic contrast-enhanced CT or MRI are APHE with washout appearance in the portal venous, delayed, or hepatobiliary phase. These criteria should be applied only to a lesion that does not show either marked T2 hyperintensity or targetoid appearance on diffusion-weighted images or contrast-enhanced images. For a second-line imaging modality, contrast-enhanced US (blood-pool contrast agent or Kupffer cell-specific contrast agent) for a “definite” diagnosis of HCC is APHE with mild and late (≥60 seconds) washout. These criteria should be applied only to a lesion that does not show either rim or peripheral globular enhancement in the arterial phase. †For diagnosis of “probable” HCC, ancillary imaging features are applied as follows. There are two categories of ancillary imaging features, those favoring malignancy in general (mild-to-moderate T2 hyperintensity, restricted diffusion, threshold growth) and those favoring HCC in particular (enhancing or non-enhancing capsule, mosaic architecture, nodule-in-nodule appearance, fat or blood products in the mass). For nodules without APHE, “probable” HCC can be assigned only when the lesion fulfills at least one item from each of the two categories of ancillary imaging features. For nodules with APHE but without washout appearance, “probable” HCC can be assigned when the lesion fulfills at least one of the aforementioned ancillary imaging features. Adopted from 2022 KLCA-NCC HCC guidelines."
pearance in hepatobiliary phases during diagnosis of HCC was a major change of radiological hallmarks in the 2018 KLCA-NCC guidelines. Since then, several studies have compared the diagnostic performance of 2018 KLCA-NCC practice guidelines to that of the Liver Imaging-Reporting and Data System (LI-RADS) and found higher sensitivity without a reduction of specificity when using MRI with liver-specific contrast. Hence, the updated 2022 KLCA-NCC guidelines retained the non-invasive diagnostic criteria of the 2018 KLCA-NCC guidelines when using MRI with liver-specific contrast. In the 2014 KLCSG-NCC guidelines, a lesion smaller than 1 cm could be non-invasively diagnosed as HCC. However, for patients who HCC developed, histologically confirmed subcentimenter-sized HCC did not fulfill the non-invasive diagnostic criteria in real-life data. The updated 2022 KLCA-NCC guidelines allow non-invasive imaging diagnosis for a nodule ≥1 cm in patients who HCC developed, In contrast, for patients with prior HCC history, the progression rate was high in patients with subcentimeter nodules showing imaging findings of HCC, allowing imaging diagnosis of recurrent HCC regardless of size.

**STAGING**

**Key recommendations**

The KLCA-NCC guidelines adopt the modified Union for International Cancer Control (mUICC) stages as the primary system, with the Barcelona Clinic Liver Cancer (BCLC) staging system and the American Joint Committee on Cancer/UICC TNM staging system serving as complements (B1).

**Real-life situation and practice**

Cancer staging plays a pivotal role in predicting prognosis, selecting treatment modality, and facilitating exchange of information. The 2022 KLCA-NCC guidelines adopted the 2003 5th version of the mUICC staging system as a primary system for HCC. For consistent analysis of registry data, the guideline committee suggested continued use of this staging system. However, the mUICC staging system has limitations, such as difficulty in exchanging information internationally. Hence, the guidelines recommended the use of other staging systems as complements.

**TREATMENT**

**Key recommendations**

Multi-disciplinary treatment has been shown to improve HCC outcome. However, the benefit, optimal frequency, format, and necessity are unknown and require further evaluation as a multi-disciplinary approach. In this situation, practice guidelines need to provide specific and practical information to clinicians when planning treatment. The KLCA-NCC 2014 guidelines began to provide best and alternative options according to mUICC stage for patients with HCC, Child-Pugh class A, no portal hypertension, and Eastern Cooperative Oncology Group (ECOG) performance status 0–1. One of the major changes to the 2022 updated guidelines addressed the quality of evidence for the best option. This will allow readers to make decisions based on increased evidence. The best option and alternative option according to mUICC stage are shown in Figure 2.

The KLCA-NCC Korea guidelines also have specific recommendations by treatment modality. Some key recommendations are shown below.

**Hepatic resection**

Hepatic resection is the primary treatment modality for single HCC limited to the liver in Child-Pugh class A patients without portal hypertension or hyperbilirubinemia (A1). Limited hepatic resection can be selectively performed for Child-Pugh class A or B7 single HCC with mild portal hypertension or hyperbilirubinemia (C1). Laparoscopic liver resection can be selectively performed for HCC located in the left lateral section and anterolateral segments (B2).

**Liver transplantation (LT)**

LT is the primary treatment modality for patients with HCC unsuitable for resection but within the Milan criteria (A1). If HCC stage is downgraded by loco-regional therapies in patients initially exceeding the Milan criteria, LT shows better outcomes than other treatments (B1). Salvage transplantation can be indicated for recurrent HCC after resection according to the same criteria used for first-line transplantation (B1).

**Local ablation therapies**

Radiofrequency ablation (RFA) has an equivalent survival
<table>
<thead>
<tr>
<th>mUIICC stage</th>
<th>Best option (quality of evidence)</th>
<th>Alternative option</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Single/≤2 cm/VI-</td>
<td>Resection (A) RFA (A)</td>
<td>cTACE TARE Other local ablation EBRT</td>
</tr>
<tr>
<td>II Single/&gt;2 cm/VI-</td>
<td>Resection (A) LT (tumor size ≤5 cm) (A) RFA (tumor size ≤3 cm) (A)</td>
<td>cTACE, TARE DEB-TACE (size &gt;3 cm) TACE+RFA (size 3-5 cm) Other local ablation (tumor size ≤3 cm) EBRT +/- TACE</td>
</tr>
<tr>
<td>III Multiple/&gt;2 cm/VI+</td>
<td>LT (within Milan criteria) (A) cTACE (A) RFA (tumor number ≤3) (B)</td>
<td>Resection (tumor number ≤3) Other local ablation (tumor number ≤3) EBRT (tumor number ≤3)</td>
</tr>
<tr>
<td>IVa Multiple/&gt;2 cm/VI+</td>
<td>1st line systemic therapy (A) cTACE+EBRT (B) cTACE (Vp1-2) (B)</td>
<td>Resection TARE EBRT</td>
</tr>
<tr>
<td>IVb Node+/no metastasis</td>
<td>1st line systemic therapy (A) cTACE+EBRT (B) cTACE (Vp1-2) (B)</td>
<td>Systemic therapy + TACE Systemic therapy + EBRT</td>
</tr>
<tr>
<td>IVb Metastasis+</td>
<td>1st line systemic therapy (A)</td>
<td>Systemic therapy + TACE Systemic therapy + EBRT</td>
</tr>
</tbody>
</table>

**Figure 2.** Best and alternative first-line treatment options in 2022 KLCA-NCC Korea guidelines for patients with HCC, Child-Pugh class A, no portal hypertension, and Eastern Cooperative Oncology Group performance status 0–1. KLCA-NCC, Korean Liver Cancer Association and National Cancer Center; HCC, hepatocellular carcinoma; mUIICC, modified Union for International Cancer Control; VI, vascular or bile duct invasion; RFA, radiofrequency ablation; cTACE, conventional transarterial chemoembolization; TARE, transarterial radioembolization; Other local ablation included percutaneous ethanol injection, microwave ablation, and cryoablation; Vp, portal vein invasion; LT, liver transplantation; DEB-TACE, drug eluting bead-TACE; TACE included cTACE and DEB-TACE; HAIC, hepatic arterial infusion chemotherapy. Adopted from 2022 KLCA-NCC HCC guidelines.
rate, a higher local tumor progression rate, and a lower complication rate compared to hepatic resection in patients with a single nodular HCC ≤3 cm in diameter (A1). Combined therapies with transarterial chemoembolization (TACE) and RFA or microwave ablation (MWA) can increase the survival rate in patients with 3–5 cm HCCs that are not amenable to hepatic resection compared to RFA or MWA alone (A2). MWA and cryoablation are expected to improve rates of survival, recurrence, and complications comparable to those of RFA (B2). Contrast-enhanced US and fusion imaging can improve the detection rate and technical success rate of local ablation therapy for HCCs ≤2 cm (B1).

**TACE and radioembolization**

Conventional TACE (cTACE) is recommended for HCC patients with a good performance status without major vascular invasion or extrahepatic spread who are ineligible for hepatic resection, LT, or local ablation therapies (A1). cTACE should be performed through tumor-feeding arteries in a superselective manner (B1). Drug-eluting bead TACE can be considered as an alternative treatment to cTACE in HCCs ≥3 cm (A2). TACE refractoriness is defined as absence of objective response (complete response or partial response), new vascular invasion, or new extrahepatic metastasis after two consecutive TACE sessions within six months; a new treatment modality should be considered in such cases (C1). cTACE alone (B2) or cTACE combined with external beam radiation therapy (EBRT) (B1) can be considered for HCC with portal vein invasion when tumors are localized within the liver and liver function is well preserved. 99mTc transarterial radioembolization (TARE) can be considered an alternative treatment to cTACE when the remnant liver function is expected to be sufficient after TARE (B2).

**EBRT**

EBRT is recommended for patients with HCC unsuitable for hepatic resection, transplantation, local ablation treatments, or TACE (C1). EBRT is performed when the liver function is Child-Pugh class A or B7 and when the volume to be irradiated with ≤30 Gy is ≥40% of the total liver volume in the computerized treatment plan (B1). EBRT can be combined for HCC expected to have an incomplete response after TACE (B2) or HCC with portal vein invasion (B2). EBRT is recommended for palliating symptoms of HCC (B1). Proton beam therapy (PBT) showed similar survival and toxicity rates when treating current or residual HCCs ≤3 cm in size (A2).

**Systemic therapies**

First-line therapies: atezolizumab plus bevacizumab or durvalumab plus tremelimumab are recommended for systemic treatment-naïve patients with locally advanced unresectable or metastatic HCC not amenable to curative or loco-regional therapy who have Child-Pugh class A with ECOG performance status 0–1 (A1). If these two combination therapies cannot be applied, sorafenib or lenvatinib is recommended (A1). Sorafenib is considered for patients with HCC who have Child-Pugh class B7 (B1) or B8–9 (B2).

Second-line therapies: the following second-line therapies can be considered or tried in patients with Child-Pugh class A and ECOG performance status 0–1 (Fig. 3).

**Adjuvant therapy**

Adjuvant immunotherapy with CIK cells can be considered after curative treatment in patients with HCC ≤2 cm without lymph node or distant metastasis (A2).

**Real-life situation and practice**

Analysis of the Korean Primary Liver Cancer Registry between 2012 and 2014 showed that various treatments were applied for the same BCLC stage. A prospective cohort study has assessed treatment patterns and outcomes of HCC patients with portal vein invasion in South Korea and found that treatment patterns are very heterogeneous without a dominant treatment modality. Many patients are being treated outside recommendations in real-life clinical practice. Major reasons for such difference between guidelines and real-life practice can be summarized as follows. First, study results with a high quality of evidence are not yet available for some clinical questions regarding management of HCC. Second, some tests, drugs, and treatments cannot be strongly recommended due to high cost or resource consumption. Third, significant disparities remain between guideline recommendation and reimbursement policy of the National Health Insurance system. South Korea has a public and single-payer system for healthcare services based on fee-for-service payments. The National Health Insurance reimbursement claim codes are used by all healthcare providers for reimbursement for their healthcare services. For patients with liver cancer, 95% of costs are reimbursed.
However, not all medical services are covered by the National Health Insurance. For some medical services, the National Health Insurance provides partial reimbursement (e.g., 50% of TARE costs are reimbursed). For some medical services (e.g., second-line treatment after atezolizumab-bevacizumab or lenvatinib in year 2022), costs are not covered by the National Health Insurance. The health insurance coverage for anti-cancer treatment has an impact on real-life practice patterns in South Korea. Fourth, there is a serious shortage of deceased donor organs. Thus, living donor liver transplantation accounts for the majority of liver transplant candidates. Living donor liver transplantation depends entirely on the discretion of the transplant team and the donor. Hence, many transplant centers consider liver transplantation even for patients with advanced HCC if a recipient has no other effective treatment options and a well-informed donor wishes to willingly participate. Fifth, substantial differences in resources or expertise for management of HCC exist according to individual medical institutions. Last, diagnostic and therapeutic methods for HCC management are among the most complicated and rapidly changing medical fields.

DISCUSSION

There are several important characteristics of the 2022 KLCA-NCC Korea guidelines. First, the guidelines adopt evidence-based recommendations by incorporating the most recent clinical data and real-world clinical practice in South Korea. Second, its recommendation regarding HCC treatment is composed of a description of individual treatment options rather than algorithm-based recommendations. Third, this guideline is a multi-disciplinary one as it is reviewed by experts in various fields of HCC management who provide various treatment modalities, including combination therapies. Fourth, the 2022 KLCA-NCC Korea guidelines suggest the best and alternative first-line treatment options for patients with HCC, Child-Pugh class A, no portal hypertension, and ECOG status 0–1. Last, since the rapidly evolving field of systemic therapy with newly approved drugs lacks robust information, expert opinion graded as level D is used to determine the optimal sequential systemic treatment in patients with advanced HCC. Although such recommendations have been reviewed by the Delphi panel of experts, they require

![Figure 3. Treatment algorithm of systemic therapies for hepatocellular carcinoma. AFP, alpha-fetoprotein. *If patients have absolute or relative contraindications for immune-checkpoint inhibitors or bevacizumab, multiple tyrosine kinase inhibitors such as sorafenib or lenvatinib should be recommended. Adopted from 2022 KLCA-NCC HCC guidelines.](https://doi.org/10.3350/cmh.2022.0404)
further improvement.

The following efforts are needed to reduce the gap between guidelines and practice for HCC management. First, studies are needed to obtain results with a high quality of evidence to answer various unresolved issues in the field of HCC management. Second, research studies should evaluate cost-benefit of tests, drugs, or treatment in South Korea. Such studies can provide a strong basis not only for clinicians, but also for policy authorities. Third, multi-disciplinary and multi-institutional studies are needed to suggest solutions for various unanswered questions. Last, we have a plan for updating guidelines when new test methods, drugs, and treatments regarding HCC are developed and new significant research findings are published. This will ultimately lead to better outcomes for HCC patients.

Authors’ contribution
Drafting of the manuscript (M.J.Goh, D.H.Sinn,M.S.Choi); Critical revision of the manuscript (all authors); Obtained funding (M.S.Choi); Study supervision (M.S.Choi); Approval of the final version of the manuscript (all authors).

Acknowledgements
This paper was supported by a grant (grant number: S-2019-2739-000) of the Research & Business Foundation of Sungkyunkwan University, Korea.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Chinese perspective

Diyang Xie1,*, Jieyi Shi1,*, Jian Zhou1,2, Jia Fan1,2, and Qiang Gao1

1Liver Cancer Institute, Zhongshan Hospital, Key Laboratory of Carcinogenesis and Cancer Invasion (Ministry of Education), Fudan University, Shanghai; 2Institute of Biomedical Sciences, Fudan University, Shanghai, China

Liver cancer is the fourth most prevalent and the second most lethal cancer in China. Hepatitis B virus (HBV) infection represents a major risk factor for hepatocellular carcinoma (HCC). Liver ultrasonography plus alpha-fetoprotein every 6 months continues to be the predominant surveillance modality. The age-Male-ALBI-Platelets score was recommended in the recent 2022 Chinese guidelines to predict HCC occurrence. The Chinese liver cancer (CNLC) staging system proposed in the 2017 guidelines continues to be the standard model for staging with modifications in the treatment allocations. Considering the aggressive nature of HBV-associated HCC, multimodal and high-intensity strategies like the addition of immunotherapy-based systemic treatment to local therapies, including resection, ablation, and intra-arterial therapies, have been adopted in real-life practices in China. The latest Chinese guidelines recommend atezolizumab plus bevacizumab, suntlimab plus a bevacizumab analog, lenvatinib, sorafenib, donafenib, and FOLFOX (folinic acid, fluorouracil, and oxaliplatin) chemotherapy as first-line treatment without priority. Regorafenib, apatinib, camrelizumab, and tislelizumab have been added as second-line systemic therapies for patients who progressed on sorafenib. Systemic therapies adopted in real-life practice are sophisticated with various combination modalities and different sequences.

(INTO Mol Hepatol 2023;29:206-216)

Keywords: Guideline; Hepatocellular carcinoma; Diagnosis; Treatment

INTRODUCTION

Liver cancer is the fourth most prevalent and the second most lethal cancer in China.1,2 Approximately 410,000 patients were newly diagnosed with liver cancer in China in 2020 (https://gco.iarc.fr), accounting for 45.3% of new global cases.3 Hepatocellular carcinoma (HCC) constitutes the majority of primary liver cancer, accounting for 75–85% of total cases. Hepatitis B virus (HBV) infection represents the major risk factor for HCC in China. According to a previous report, 69.9% of Chinese patients with HCC had a background of HBV infection, 5.2% had hepatitis C virus (HCV) infection, and 5.8% had both.4 Other risk factors include aflatoxin exposure, alcohol abuse, and metabolic disorders. Since 1992, the neonatal HBV vaccination program5 and effective anti-viral agents have contributed to a significant decline in HCC inci-
idence, especially for those below 40 years old. HBV-associated HCC belongs to a molecular subtype named proliferation subtype and is featured by poor differentiation and high aggressiveness. According to the BRIDGE (Bridge to Better Outcomes in HCC) study, only 36% of Chinese cases were initially diagnosed at the early stage and eligible for curative treatments, while the remnant 9% and 55% were at an intermediate and advanced stage, respectively. Efforts to improve the diagnostic sensitivity and the therapeutic efficacy of HCC treatment have contributed to a decrease of 20.3% in age-standardized mortality in China from 1990 to 2017.

Evidence-based clinical guidelines on the management of HCC have been updated every two to three years by a multidisciplinary group of experts in China. Herein, we present a concise review of the latest version (2022 version) and discuss real-life practices in China.

SURVEILLANCE

Patients with chronic HBV/HCV infection, cirrhosis of any causes, alcohol abuse, non-alcoholic steatohepatitis, or family history of HCC are listed as a high-risk population for developing HCC, especially among men over 40 years. The age-Male-ALBI-Platelets (aMAP) score has been added to the 2022 Chinese guidelines to help discriminate a high-risk population for HCC occurrence (aMAP score >60 with an annual HCC incidence reaching 1.6–4%). For these high-risk populations, ultrasonography (US) plus alpha-fetoprotein (AFP) should be carried out every 6 months.

Considering the social cost-effectiveness in a country with a large HBV-infected population, liver US plus AFP every 6 months continues to be the predominant surveillance modality in China. In Japan where over 70% of HCC patients are initially diagnosed at an early stage, liver US plus AFP, des-gamma-carboxy prothrombin, and AFP-L3 testing are performed every 6 months for high-risk groups (HBV/HCV infection, cirrhosis of other etiologies) and every 3–4 months for extremely high-risk groups (HBV/HCV related cirrhosis). Apart from insufficient testing items and surveillance frequency, the lack of government-supported programs to cover surveillance for more populations also contributes to a relatively low diagnostic rate of early HCC in China. The integration of resources from the community into the hospital to establish an efficient surveillance and call-back system is expected to resolve such a dilemma. In addition, new blood-based biomarkers including a 7-miRNA panel and GALAD (gender, age, AFP-L3, AFP, DCP) score have been developed to assist in the early detection of HCC, especially for AFP-negative patients.

DIAGNOSIS

The diagnosis of HCC can be made pathologically or via typical imaging hallmarks. For patients with liver cirrhosis or chronic hepatitis B/C, nodules \( \geq 2 \) cm can be diagnosed as HCC based on the typical features of arterial phase hyper-enhancement (APHE). These include APHE with washout appearance at the portal venous or delayed or hepatobiliary phases on any of the three imaging modalities including multiphasic dynamic computed tomography (CT), dynamic magnetic resonance imaging (MRI), or gadolinium-ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced MRI (EOB-MRI). In addition, diagnosis can be based on the typical features of APHE with late (≥60 seconds) washout appearance in the Kupffer phase on contrast-enhanced ultrasound (CEUS). On the other hand, the diagnosis of nodules \( \leq 2 \) cm can be established when the typical features are present on at least two imaging modalities. Otherwise, a biopsy is recommended in case of an inconclusive diagnosis.

Nodules \( \geq 1 \) cm can be diagnosed as HCC with typical hallmark on a single imaging technique in Japan, Korea, and western countries, whereas the diameter \( \geq 2 \) cm is set as a
prerequisite for the diagnosis via only one imaging modality in China. In fact, for nodules of 1–2 cm, typical features on both dynamic CT and MRI used to be required for a definite diagnosis of HCC in western countries. However, a decreased sensitivity with limited improvement in diagnostic specificity via coincidental dynamic CT plus MRI in later studies reaffirmed the application of a single modality to diagnose lesions ≥1 cm by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver (EASL). Considering the unbalanced distribution of medical resources in China, diagnostic accuracy is given priority over sensitivity. Therefore, confirmation via two imaging techniques for nodules of 1–2 cm continues to be adopted in China. EOB-MRI and CEUS have been included as diagnostic modalities to increase diagnostic sensitivity in China since 2017.

STAGING

The China Liver Cancer Staging (CNLC) system (Fig. 1), which incorporates tumor characteristics, liver function, and performance status, similar to the Barcelona Clinic of Liver Cancer (BCLC) system, was established in 2017 and has been adopted ever since. Concerning tumor status, each stage of BCLC 0/A, B, and C is divided into two substages in the CNLC system, including stages Ia, Ib, Ila, IIa, IIIa, and IIIb. CNLC stage IV is equivalent to BCLC stage D.

Similar to the BCLC system, the CNLC system is a treatment allocation method for decision-making purposes, whereas the Japan Integrated Staging score and its variants focus on the prognostic predictive function. The modified Union for International Cancer Control system adopted in Korea is characterized by more detailed treatment allocation and is applied on the premise of the Child-Pugh A function, no portal hypertension, and performance score (PS) scoring 0–1. The CNLC system has been in wide use in real-life practices, although the BCLC system continues to be the main stratification factor for clinical trial designing.

Figure 1. The CNLC staging and treatment algorithms of HCC. CNLC, Chinese liver cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; PS, performance score; MDT, multi-disciplinary treatment; UCSF, university of California San Francisco.
TREATMENTS

Hepatectomy

Hepatectomy is preferably indicated for patients with CNLC stage Ia, Ib, and IIA HCC. For patients with CNLC IIB and IIA HCC who are optimal candidates for transarterial chemoembolization (TACE) and systemic therapy, respectively, surgical resection can be considered if tumor nodules are localized in the same segment or lobe, and tumor emboli are expected to be completely resected. Child-Pugh grade A, an indocyanine green (ICG) 15-minute retention rate <30%, and future remnant liver volume accounting for more than 40% (for patients with liver fibrosis/cirrhosis) or more than 30% (for patients without liver fibrosis/cirrhosis) are prerequisites for hepatectomy. Minimally invasive laparoscopic or robot-assisted laparoscopic liver resection (LLR) is recommended in experienced centers.

In real-life practice, conversion therapy via multimodal and high-intensity anti-tumor strategies is advocated to improve resectability and long-term survival for patients with potentially resectable HCC, defined as technically unresectable CNLC stage Ia, Ib, IIA HCC, or technically resectable IIB, IIA HCC. Systemic therapies like tyrosine kinase inhibitors (TKIs) plus programmed death-1 (PD-1) inhibitors and locoregional treatments like hepatic arterial infusion chemotherapy (HAIC) plus TACE have been explored as conversion therapies to induce tumor shrinkage or downstaging. As effective methods to introduce liver regeneration for patients with unmet future liver reserve, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) were deemed to be superior to portal vein embolization in the faster introduction of liver regeneration and fewer risks of tumor progression. For patients who failed to achieve sufficient hypertrophy after conventional ALPPS stage-1 due to severe fibrosis/cirrhosis, transcatheter arterial embolization-salvaged ALPPS (TAE-salvaged ALPPS) is a new strategy to increase the resectability of HCC.

Lesions that are less than 10 cm and located in Couinaud segments II, III, IVb, V, and VI without affecting the anatomy of the first and second hepatic hilus used to be the best candidates for LLR. With the development of minimally invasive techniques, especially the use of ICG fluorescence, indications for LLR are expanded without strict restrictions on tumor size and tumor location in an experienced center in China. Although propensity score-matched (PSM) studies have affirmed comparable oncologic outcomes between LLR and open resection, especially for early-stage HCC, multi-center, randomized, controlled studies are warranted to validate the equal long-term efficacy of LLR compared to open resection.

Transplantation

The University of California San Francisco criteria (solitary tumor ≤6.5 cm or ≤3 nodules ≤4.5 cm plus total tumor diameter ≤8 cm) continues to be advocated as the major criteria for liver transplantation (LT) in the latest Chinese guideline. For patients who are initially beyond the LT criteria, downstaging therapies via locoregional therapies are recommended to reduce tumor burden to be within the LT criteria. Early withdrawal of or no corticosteroid and replacement of calcineurin inhibitors with mammalian target of rapamycin (mTOR) inhibitors are recommended to prevent tumor recurrence after LT.

In real-life practices, a number of criteria have been adopted in different centers, including Shanghai Fudan criteria, West China criteria, and Sanya consensus with minor differences in tumor number and tumor size. Although transplant patients are usually excluded from immune checkpoint inhibitor therapy for fear of possible graft rejection, experience in our center showed that grafts without PD-L1 expression seemed to represent a useful marker for not developing graft-related immune-related adverse events, the result of which needs confirmation by more studies.

Ablation

Consistent with previous versions of clinical guidelines, ablation as a curative approach is indicated for CNLC Ia and Ib HCC (single nodule ≤5 cm, 2–3 nodules with each ≤3 cm). Radiofrequency ablation (RFA) and microwave ablation (MWA) are recommended equally without priority. For unresectable single HCC with a diameter of 3–7 cm, ablation in combination with TACE is recommended.

In real-life practices in China, ablation is not only applied to early HCC, its combination with TACE has also been implemented in patients with intermediate HCC. After PSM, MWA plus TACE yielded superior progression free survival (PFS) and overall survival (OS) to TACE alone for BCLC stage B HCC.
Notably, the morphological criteria and treatment modality varied among different studies. Zhang et al. included patients with 2–5 tumor nodules with each ≤7 cm as a target population for MWA in combination with TACE, whereas Li et al. enrolled patients with either single tumors ≤8 cm or 2–5 tumors each ≤5 cm. The above studies adopted a sequential treatment of MWA after TACE at an interval of at least one month, another study proposed a concurrent treatment of TACE and MWA. On the other hand, although the role of PD-1 inhibitors in the adjuvant setting is still under investigation, promising results in early-stage clinical trials have encouraged the administration of PD-1 blockade in addition to RFA in real-life practices. A retrospective study demonstrated that PD-1 inhibitors in addition to RFA for recurrent HCC resulted in a significantly improved 1-year recurrence-free survival (RFS) rate compared to RFA alone.

**INTRA-ARTERIAL THERAPIES**

TACE has been mainly indicated for CNLC IIb, IIIa, and some IIIb HCC since the 2017 Chinese guidelines. For patients with CNLC Ia, Ib, and IIa HCC ineligible for curative treatments, TACE is recommended as an alternative. Conventional TACE (cTACE) was equally recommended with drug-eluting bead-TACE due to similar OS benefits. Super-selective TACE with the assistance of Cone-Beam CT if necessary is recommended to guarantee the efficacy of TACE. cTACE is also recommended in the adjuvant setting for patients with high recurrent risks including multiple lesions, evidence of tumor thrombus or tumor diameter >5 cm.

In real-life practices, the combination of TACE with other locoregional treatment or systemic therapy is emphasized. Optimizing the management of intermediate-stage HCC has been a research hotspot. Although sorafenib in combination with TACE yielded a similar OS to TACE alone for intermediate HCC, the improved PFS provided by sorafenib and the prolonged OS rendered by lenvatinib supported the addition of TKIs to TACE. Triple therapy of TACE integrated with TKIs plus PD-1 inhibitor for intermediate HCC showed favorable efficacy in controlling tumor progression and provided an opportunity for resection. On the other hand, when TACE is indicated for HCC patients with portal vein invasion or extrahepatic metastasis in case collateral compensation exists or extrahepatic tumor burden is limited, the addition of systemic therapy such as sorafenib, lenvatinib, or lenvatinib plus PD-1 antibody is a routine practice in some institutions and demonstrated with more favorable tumor control than systemic therapy alone. Y90 transarterial radioembolization (TARE) has not been widely applied in China currently.

While HAIC using interferon, cisplatin, or low-dose 5-FU plus cisplatin regimen alone or in combination with sorafenib was not recommended for advanced HCC due to negative results in Japan, HAIC using FOLFOX (folinic acid, fluorouracil, and oxaliplatin) regimen developed in China was not only adopted as an alternative treatment for TACE-refractory or TACE-unsuitable patients but also as a first-line treatment for advanced HCC due to prolonged median OS compared to sorafenib for advanced HCC. For patients with large unresectable HCC (largest diameter ≥7 cm) without macrovascular invasion or extrahepatic spread, FOLFOX-HAIC also significantly improved OS compared to TACE. Moreover, the strong antitumor efficacy for intrahepatic lesions enabled FOLFOX-HAIC as a conversion therapy to transform HCC from unresectable to resectable. A recent study reported that 1–2 cycles of FOLFOX-HAIC in the adjuvant setting significantly improved RFS for patients with microvascular invasion. Although FOLFOX-HAIC has not been clearly recommended in the current guidelines, it is accepted as an effective locoregional treatment modality across all stages of HCC in China. Nonetheless, there still lacks a consensus on the treatment modality of FOLFOX-HAIC in different regimes of FOLFOX (oxaliplatin, 85 or 130 mg/m² for 2 hours; leucovorin, 400 mg/m²; fluorouracil bolus 400 mg/m², and 5-fluorouracil, 2,400 mg/m² for 46 hours or 2,400 mg/m² for 24 hours or 1,200 mg/m² for 22 hours) were administered in different studies. Moreover, the addition of systemic therapies including target agents and immunotherapy to HAIC turned out to improve overall response rate, although the long-term effects on liver function and OS need to be clarified in the future.

**RADIOThERAPY**

Stereotactic body radiotherapy is indicated for patients with CNLC Ia and some Ib HCC who are ineligible for curative treatments or who are reluctant to receive invasive treatment. For patients with CNLC IIa or IIb HCC, external beam radiation therapy in combination with TACE can improve local tumor control. For patients with resectable CNLC IIIa HCC,
external ablation can be performed to control tumor thrombus in the neoadjuvant or adjuvant setting to prolong OS.\(^6\)

In real-life practices, external beam radiotherapy is carried out with recommendations in the guidelines. As for internal radiation, Y90 TARE has not been applied widely in China. On the other hand, TACE in combination with \(^{125}\)I seed and stent implantation has been implemented for patients with type II tumor thrombus and demonstrates superior survival benefits to TACE alone.\(^6\)

### SYSTEMIC THERAPY

Systemic therapy is mainly indicated for patients with advanced HCC, namely CNLC IIIa and IIb patients. For patients who had CNLC IIb HCC ineligible for locoregional therapies or who are refractory to TACE, transition to systemic therapy is recommended and covered by Chinese national reimbursement. The current Chinese guidelines recommend atezolizumab plus bevacizumab (Atezo-Bev),\(^7\) sintilimab plus bevacizumab analog (Byvasda),\(^8\) lenvatinib, sorafenib, donafenib,\(^6\) and FOLFOX chemotherapy as the first-line treatment without priority. Apart from regorafenib, targeted agent apatinib and PD-1 inhibitors camrelizumab\(^9\) and tislelizumab\(^10\) have been added as second-line systemic therapies for patients who have progressed on sorafenib. Cabozantinib\(^11\) and ramucirumab,\(^12\) which are approved as second-line treatments for selected patients in other countries, have not been marketed in China.

Since 2017, with the approval of new first-line and second-line agents, the treatment options for advanced HCC are more diverse than ever. Apart from PD-1/PD-L1 inhibitor plus vascular endothelial growth factor receptor (VEGFR) inhibitor including Atezo-Bev and sintilimab-Byvasda, PD-1/PD-L1 inhibitors plus TKIs have been in wide use in real-life practices in China. The RESCUE trial, evaluating the efficacy of camrelizumab plus apatinib versus sorafenib as first-line therapy, met the dual primary endpoint and showed significant improvements in OS (22.1 vs. 15.2 months, \(P<0.001\)) and PFS (5.6 vs. 3.7 months, \(P<0.001\)) in the combination arm.\(^13\) Although lenvatinib plus pembrolizumab versus lenvatinib monotherapy failed to meet pre-specified statistical significance in OS (21.2 vs. 19.0 months, \(P=0.233\)) in the phase III LEAP-002 study,\(^14\) the clinically meaningful survival benefit still provided a rationale for its use as first-line treatment. Sorafenib\(^15\) or lenvatinib\(^16,17\) plus PD-1 inhibitor as first-line treatment and regorafenib plus PD-1 inhibitor\(^18,19\) as second-line treatment have shown promising survival benefits with manageable toxicity in Chinese patients. Nevertheless, the insignificant OS improvement of cabozantinib plus atezolizumab versus sorafenib (15.4 vs. 15.5 months, \(P=0.440\)) in the COSMIC-312 study suggested that immunotherapy in addition to targeted agents did not guarantee synergistic efficacy in the clinic.\(^20,21\)

Although dual immunotherapies of durvalumab plus tremelimumab\(^22\) in the first-line setting and nivolumab plus ipilimumab\(^23,24\) in the second-line setting approved in western countries have not been included in current Chinese guidelines, their applications as post-line therapies after TKI failure, PD-1/PD-L1 monotherapy failure or TKI plus PD-1/PD-L1 combination therapy failure are adopted in real-life practice. Further evidence for dual immunotherapy as post-line therapy is needed. On the other hand, the current approved second-line agents indicated for patients who progressed on sorafenib can apply equally to those who progressed on lenvatinib. According to a retrospective study, lenvatinib-regorafenib sequential therapy yielded prolonged total OS (29.7 vs. 23.0 months, \(P=0.041\)) and post-regorafenib OS (15.9 vs. 11.7 months, \(P=0.045\)) compared to sorafenib-regorafenib sequential therapy.\(^9\) With the advent of more effective treatments, the optimal sequence or a combination modality suitable for individual patients needs to be clarified by high-evidence trials.

### DISCUSSION

To provide useful and accessible information for all clinicians, the Chinese guidelines on the management of HCC incorporated literature based on not only randomized clinical trials (RCT) but also observational studies of newly developed clinical practices with levels of evidence classified by revised Grading of Recommendations, Assessment, Development and Evaluation.\(^25\) Novel findings on the surveillance, diagnosis, and treatment of HCC are elaborated in the appendix of updated guidelines for a deep understanding of new trends.

Considering the aggressive nature of HBV-associated HCC, multimodal treatments like the addition of immunotherapy-based systemic treatment to local therapies have been adopted in real-life practices in China. The development of original drugs, such as apatinib, sintilimab, camrelizumab,
and tislelizumab, and biologically similar drugs, such as Byvasda, in China, enable patients to accept the aforementioned multimodal treatment with an affordable economic burden. Nevertheless, with regard to social cost-effectiveness, only treatment with high-grade evidence-based RCTs is covered by insurance, which somehow narrows the gap from the optimal guideline recommendations to real-life practices at the government level. Moreover, more than 20 phase III trials are in progress to identify the role of ICI-based therapies across all stages of HCC. The release of these results in the next 5 years should lead to a consensus on the addition of immunotherapy-based systemic therapy to HCC at different stages.

Authors’ contribution
Drafting of the manuscript (Diyang Xie, Jieyi Shi); Critical revision of the manuscript for important intellectual content (all authors); Obtained funding (Jieyi Shi, Qiang Gao); Study supervision (Qiang Gao).

Acknowledgements
This work was supported by National Natural Science Foundation of China (No. 82130077, 82090053 and 81961128025), Basic Research Project from the Science and Technology Commission of Shanghai Municipality (Grants 21JC1410100, 21JC1401200, 20JC1418900).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


cinoma 2021;8:1233-1240.


Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Hong Kong perspective

Rex Wan-Hin Hui, Lung-Yi Mak, Tan-To Cheung, Victor Ho-Fun Lee, Wai-Kay Seto, and Man-Fung Yuen

1Department of Medicine, School of Clinical Medicine, The University of Hong Kong, Hong Kong; 2State Key Laboratory of Liver Research, The University of Hong Kong, Hong Kong; Departments of 3Surgery and 4Clinical Oncology, School of Clinical Medicine, The University of Hong Kong, Hong Kong

Hepatocellular carcinoma (HCC) is a major public health burden in Hong Kong, and chronic hepatitis B is the most common HCC etiology in our region. With the high case load, extensive local expertise on HCC has been accumulated. This article summarized local guidelines and real-life practice on HCC management in Hong Kong. For HCC surveillance, liver ultrasound and serum alpha-fetoprotein for periodic screening is recommended in viral hepatitis or cirrhotic patients, and this is adhered to in clinical practice. HCC diagnosis is not covered in local guidelines, yet our practice is in-line with regional guidelines, where diagnosis is usually achieved by cross-sectional imaging and without the need for histology. Our guidelines recommend using the Hong Kong Liver Cancer Staging for pre-treatment staging, yet we routinely use other widely-adopted systems such as the Barcelona Clinic Liver Cancer Staging and the Tumor-Node-Metastasis Staging as well. Our local guidelines have provided clear treatment algorithms for the whole range of HCC therapies, including resection, ablation, transplant, transarterial chemoembolization, transarterial radioembolization, stereotactic body radiation therapy, targeted therapy, and immunotherapy. Real-life treatment choices are largely in line with the guidelines, although treatment protocols are individualized, and availability of specific therapies can vary between centers. Overall, HCC guidelines in Hong Kong are tailored based on local expertise and our unique patient population. The guidelines are up-to-date and provide practical pathways to assist our routine practice. Regular updates of local guidelines are warranted to account for the rapidly evolving paradigm of HCC management. (Clin Mol Hepatol 2023;29:217-229)

Keywords: Hepatocellular carcinoma; Hepatitis B; Hepatectomy; Liver transplantation; Immunotherapy
INTRODUCTION

Liver cancer is the fifth most common cancer and the third leading cause of cancer death in Hong Kong. Among primary liver cancers, hepatocellular carcinoma (HCC) is the major type which accounts for 90% of cases. From 1992 to 2006, around 80% of HCC in Hong Kong were attributable to chronic hepatitis B (CHB), and only 6.3% were attributable to chronic hepatitis C (CHC). This contrasts with the key HCC risk factors of CHC, non-alcoholic steatohepatitis (NASH), and alcoholic liver disease in Western countries.

CHB-related HCC patients generally have better preserved liver function than HCC patients with other liver diseases, as hepatitis B virus (HBV) is directly oncogenic and can induce HCC in non-cirrhotic patients. Furthermore, given the high case load and experience in HCC management, experts in Hong Kong generally propose more aggressive treatment approaches than that recommended in international guidelines.

This article will discuss both the local guidelines and real-life practice on HCC care in Hong Kong, covering the areas of screening, diagnosis, staging and treatment (Table 1). The management framework of HCC in Hong Kong is depicted in Figure 1. The key guidelines covered include (1) Recommendations on Prevention and Screening for Liver Cancer for Health Professionals (Published in 2018); (2) The Hong Kong Liver Cancer (HKLC) Staging system (Published in 2014); (3) The Hong Kong Consensus Statement on the Management of Hepatocellular Carcinoma (Hong Kong Consensus) (Published in 2022); and (4) Systemic Treatment of Advanced Unresectable Hepatocellular Carcinoma after First-Line Therapy: Expert Recommendations from Hong Kong, Singapore, and Taiwan (Published in 2022).

SURVEILLANCE

Clinical guidelines

The Hong Kong Cancer Expert Working Group on Cancer Prevention and Screening published recommendations on HCC screening in 2018. The guidelines recommend the combined use of liver ultrasound (USG) and serum alpha-fetoprotein (AFP) for HCC screening, whereas computed tomography (CT) and magnetic resonance (MR) scans are recommended in patients with suboptimal USG assessment. CHB, CHC and cirrhotic patients are recommended to receive period screening (every 6–12 months), depending on age, family history and other clinical parameters.

Real-life practice

Patients with chronic liver diseases are usually followed-up by hepatologists in Hong Kong, and eligible patients are advised for screening at 6-month intervals. Local data has demonstrated that screening by USG and/or AFP is associated with HCC detection at earlier stages and improved survival.

The availability of CT and MR scans is increasing in our locality, and patients with suboptimal USG assessment (such as in obesity, ascites or high-lying liver) are referred for CT or MR. The use of artificial intelligence on CT scans and advanced MR imaging metrics have also been explored as surveillance options in exploratory studies in Hong Kong.

DIAGNOSIS

Clinical guidelines

No local guidelines have focused on HCC diagnosis in Hong Kong, and Asia-Pacific regional guidelines are frequently referenced to by clinicians. The Asia-Pacific guidelines recommend triphasic CT or gadoxetic acid-enhanced MR for diag-

Abbreviations:
AFP, alpha-fetoprotein; ALPPS, associating liver partition and portal vein ligation for staged hepatectomy; BCLC Staging, Barcelona Clinic Liver Cancer Staging; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CT, computed tomography; DDLT, deceased-donor liver transplant; DEB-TACE, TACE with drug-eluting beads; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIFU, high-intensity focused ultrasound; HKLC Staging System, Hong Kong Liver Cancer Staging System; ICG, indocyanine green; LDLT, living-donor liver transplant; LI-RADS, Liver Reporting & Data System; MELD, model for end-stage liver disease; MR, magnetic resonance; MWA, microwave ablation; NASH, non-alcoholic steatohepatitis; PET-CT, positron emission tomography-computed tomography; PVE, portal vein embolization; RFA, radiofrequency ablation; SBRT, stereotactic body radiation therapy; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; TKI, tyrosine kinase inhibitor; TNM Staging, Tumor-Node-Metastasis Staging; UCSF, University of San Francisco; USG, ultrasound
Table 1. Summary of clinical guidelines and real-life practice for hepatocellular carcinoma in Hong Kong

<table>
<thead>
<tr>
<th>Management</th>
<th>Guidelines</th>
<th>Real-life practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surveillance</td>
<td>Liver USG+serum AFP at 6–12 month intervals in CHB/CHC/Cirrhosis CT/MR in patients with suboptimal USG assessment</td>
<td>Liver USG+serum AFP at 6-month intervals for at-risk patients CT/MR in patients with suboptimal USG assessment</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>No local guidelines available</td>
<td>Triphasic CT widely available and is the most commonly utilized test</td>
</tr>
<tr>
<td></td>
<td>Asia-Pacific guidelines frequently referenced: Triphasic CT or Gadoxetic acid-enhanced MR for diagnosis; histology not required if imaging features typical</td>
<td>Gadoxetic acid-enhanced MR is rising in popularity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dual-tracer PET-CT is rising in popularity as a confirmatory diagnostic test plus staging test. Its use is supported by local data</td>
</tr>
<tr>
<td>Staging</td>
<td>HKLC Staging System</td>
<td>HKLC Staging is used, although traditional staging systems (BCLC Staging/TNM System) are still frequently used as well</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatment decisions individualized in multidisciplinary management</td>
</tr>
<tr>
<td>Curative treatment</td>
<td>Resection is first-line treatment if liver function satisfactory and anatomically resectable</td>
<td>Resection is first-line, with pre-hepatectomy workup by ICG retention testing and CT volumetry assessment</td>
</tr>
<tr>
<td></td>
<td>Resection in HCC with intrahepatic portal vein invasion, limited extrahepatic metastasis and bilobal disease may be performed in specialized centers</td>
<td>PVE and ALPPS available, although ALPPS only available in specialized centers</td>
</tr>
<tr>
<td></td>
<td>PVE or ALPPS to enhance resectability in patients with inadequate future liver remnant</td>
<td>Hepatectomy in HCC with portal vein thrombosis is performed, although disease recurrence rates are high (&gt;80%)</td>
</tr>
<tr>
<td></td>
<td>Local ablation (RFA/MWA/HIFU/Cryoablation) is an alternative in Child-Pugh A/B patients with HCC &lt;3 cm</td>
<td>Combined resection+RFA of multifocal HCC is performed, with improved short-term morbidity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resection of lung metastasis after hepatectomy is performed, with survival benefits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RFA is frequently performed, and local data shows comparable recurrence and 10-year overall survival when comparing RFA with resection for early-stage HCC</td>
</tr>
<tr>
<td>Liver transplant</td>
<td>Patient selection by UCSF Criteria</td>
<td>Single transplant center in the region that performs both DDLT and LDLT</td>
</tr>
<tr>
<td></td>
<td>Local ablation/TACE/TARE/SBRT are suitable bridging options for waitlisted patients</td>
<td>Cadaveric grafts allocated based on MELD, with adjustment for HCC status</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDLT is the predominant type of transplant performed, with favorable outcomes in local data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TACE and SBRT are the most commonly performed bridging therapies</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>No local guidelines available</td>
<td>Post-treatment surveillance by CT/MRI+serum AFP every 6 months for 2 years, followed by surveillance at 6–12 month intervals thereafter</td>
</tr>
<tr>
<td>surveillance</td>
<td></td>
<td>Antivirals to reduce recurrence risk in patients with CHB</td>
</tr>
</tbody>
</table>
Management

<table>
<thead>
<tr>
<th>Non-curative treatment</th>
<th>Loco-regional therapy</th>
<th>Systemic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACE recommended in unresectable HCC with no vascular invasion or extrahepatic spread and satisfactory liver function</td>
<td>Segmental portal vein thrombosis and venous invasion are not absolute contraindications for TACE</td>
<td>First line options: Atezolizumab+Bevacizumab, Nivolumab monotherapy, TKIs (Lenvatinib or Sorafenib)</td>
</tr>
<tr>
<td>SBRT and TARE are suitable for Child-Pugh A patients, especially if HCC &gt;5 cm</td>
<td>DEB-TACE is available if not responsive to TACE</td>
<td>First-line treatment option chosen based on liver function, potential contraindications, and patient preferences</td>
</tr>
<tr>
<td>TACE is first-line in patients with unresectable HCC</td>
<td>SBRT +/- TACE is performed, local data shows SBRT+TACE leads to improved survival when compared with TACE alone</td>
<td>Second-line options: Regorafenib, Cabozantinib, Ramucirumab</td>
</tr>
<tr>
<td>DEB-TACE available in specialized centers</td>
<td>TARE less frequently performed than TACE, although limited data suggest comparable short-term survival with TACE and TARE</td>
<td>Second-line immunotherapy considered in patients who have failed first-line TKIs</td>
</tr>
<tr>
<td>SBRT and TACE are performed, local data shows SBRT+TACE leads to improved survival when compared with TACE alone</td>
<td>TARE less frequently performed than TACE, although limited data suggest comparable short-term survival with TACE and TARE</td>
<td>No clear instructions on patients who progress beyond second-line</td>
</tr>
</tbody>
</table>

Systemic therapy

<table>
<thead>
<tr>
<th>Systemic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>First line options: Atezolizumab+Bevacizumab, Nivolumab monotherapy, TKIs (Lenvatinib or Sorafenib)</td>
</tr>
<tr>
<td>First-line treatment option chosen based on liver function, potential contraindications, and patient preferences</td>
</tr>
<tr>
<td>Second-line options: Regorafenib, Cabozantinib, Ramucirumab</td>
</tr>
<tr>
<td>Second-line immunotherapy considered in patients who have failed first-line TKIs</td>
</tr>
<tr>
<td>No clear instructions on patients who progress beyond second-line</td>
</tr>
</tbody>
</table>

TACE, transarterial chemoembolization; TARE, transarterial radioembolization; TKI, tyrosine kinase inhibitor; TNM Staging, Tumor-Node-Metastasis Staging; UCSF, University of San Francisco; USG, ultrasound.
Real-life practice

Imaging is the predominant modality for HCC diagnosis in Hong Kong, and histology is rarely required. Triphasic CT is available in most tertiary hospitals in our locality, and it is the most frequently utilized test for HCC diagnosis. Nonetheless, gadoxetic acid-enhanced MR has risen in popularity in recent years for its higher sensitivity and better lesion delineation.

Dual-tracer positron emission tomography-CT (PET-CT) with 11C-Acetate and 18F-fluorodeoxyglucose is another increasingly popular diagnostic test for HCC in our locality. Dual-tracer PET-CT is usually used as a confirmatory test in patients with indeterminate findings on CT or MR. Local data has demonstrated that dual-tracer PET-CT is significantly less affected by cirrhotic changes than contrast CT for HCC diagnosis. Furthermore, dual-tracer PET-CT has the additional benefit of detecting metastatic foci, aiding the disease staging process. A cost-effectiveness study using local data demonstrated that dual-tracer PET-CT is cost-effective for assessment in patients with AFP ≥400 ng/mL or with bilobar disease.

STAGING

Clinical guidelines

The HKLC Staging System is recommended as the staging system of choice in the Hong Kong Consensus Guidelines. The HKLC Staging was developed in 2014 with data from 3,856 HCC patients in Hong Kong. The rationale for developing the HKLC Staging was to account for the high HBV prevalence and generally more aggressive treatment approach in Hong Kong. The prognostic factors in the HKLC Staging include Eastern Cooperative Oncology Group status, Child-Pugh score, presence of extravascular invasion or metastasis, tumor size and number of tumor nodules (Fig. 2).

Real-life practice

After the diagnosis of HCC in Hong Kong, patients are referred from hepatologists to specialized HCC clinics, where they receive joint care by surgeons and oncologists to individualize their next step of treatment. Hepatologists will regularly review the patients and advise on management of the primary liver disease (e.g., antiviral use) and on cirrhotic complications (e.g., diuretic titration, variceal screening). However, the anti-cancer therapy decisions including planning for surgical therapy, TACE, or systemic therapy are primarily handled by surgeons and oncologists.

When comparing the HKLC Staging with the widely adopted Barcelona Clinic Liver Cancer (BCLC) Staging, HKLC more clearly delineates intermediate or advanced stage patients into smaller subgroups, enabling different treatment strategies for this heterogenous patient group. Models have shown HKLC to have better prognostic performance over other staging systems in Hong Kong. Nonetheless, traditional staging systems such as the BCLC Staging and the Tumor-Node-Metastasis System remain frequently used in clinical practice and research in Hong Kong, possibly due to historical reasons and for ease of communication.
**CURATIVE TREATMENT – RESSECTION AND ABLATION**

**Clinical guidelines**

The Hong Kong Consensus recommends liver resection as the first-line treatment for HCC with solitary lesion or with several lesions limited to segment(s) with resectable potential, given satisfactory liver function reserve. For patients with inadequate future liver remnant, portal vein embolization (PVE) or associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) are both suitable to enhance resectability.

Portal vein invasion, extrahepatic metastasis and bilobar disease are traditionally listed as contraindications for hepatectomy in Western guidelines, whereas the Hong Kong Consensus recommends consideration of hepatectomy in highly-selected patients. For patients with intrahepatic portal vein or hepatic vein branch invasion, resection can be considered in specialized centers. For limited extrahepatic metastasis and bilobar disease, the predominant HCC lesion can be resected, while the extrahepatic metastasis or contralateral liver lobe nodules can be managed by resection or ablation. For limited extrahepatic metastasis and bilobar disease, the predominant HCC lesion can be resected, while the extrahepatic metastasis or contralateral liver lobe nodules can be managed by resection or ablation. For limited extrahepatic metastasis and bilobar disease, the predominant HCC lesion can be resected, while the extrahepatic metastasis or contralateral liver lobe nodules can be managed by resection or ablation.

Local ablation is recommended as an alternative to resection in Child-Pugh A/B patients with HCC <3 cm. The options of radiofrequency ablation (RFA), microwave ablation (MWA),...
high-intensity focused ultrasound (HIFU), and cryoablation are all suitable, whereas percutaneous ethanol injection is less preferred due to lower complete ablation rates and higher recurrence rates.5

**Real-life practice**

In Hong Kong, liver resection is the main treatment option for early-stage HCC. The pre-hepatectomy workup involves indocyanine green retention testing and CT volumetry assessment.21 For patients with inadequate future liver remnant, ALPPS or PVE may be offered, although the availability of ALPPS is limited and is only performed in specialized centers. A local study conducted in 2021 reported significantly higher resection rates for ALPPS when compared with PVE (97.8% vs. 67.7%), although the two methods conferred comparable short-term and long-term mortality.21

Hepatectomy in patients with portal vein thrombosis has been performed for over three decades in Hong Kong. The median disease-free survival ranged from 1.5 to 4.2 months, and overall survival ranged from 8.6 to 10.9 months.22 These recurrence and mortality rates are in line with data from other regions.23

Combined resection and intraoperative RFA for multifocal HCC have also been performed in Hong Kong. Combined resection and RFA, when compared with resection alone, led to fewer major resections, less blood loss, shorter operation time and shorter hospital stay, with no differences in recurrence and overall survival.24 Local data is also available for patients with resectable HCC with lung metastasis, and successful resection of lung metastasis after hepatectomy is associated with significant improvement in survival.25

A randomized trial in Hong Kong performed head-to-head comparison between RFA and resection for early-stage HCC. RFA led to shorter treatment duration, shorter hospital stay, and lower blood loss, although the recurrence rate and 10-year overall survival were comparable.26 RFA is feasible and frequently considered in Hong Kong for patients with high surgical risk. Other ablation options such as MWA and cryoablation are less frequently performed locally.

---

**CURATIVE TREATMENT – LIVER TRANSPLANT**

**Clinical guidelines**

Liver transplantation is recommended by the Hong Kong Consensus in patients with poor liver function, as it can treat both HCC and cirrhosis.5 The guidelines recommend patient selection for transplant by the University of California San Francisco criteria – Solitary tumor <6.5 cm; or <3 tumor nodules with largest lesion <4.5 cm with total tumor diameter <8.0 cm.27

For patients waitlisted for transplant, options of bridging therapy include local ablation, transarterial chemoembolization (TACE), transarterial radioembolization (TARE) and stereotactic body radiation therapy (SBRT).5

**Real-life practice**

Our hospital (Queen Mary Hospital, Hong Kong) is the only designated liver transplant center in Hong Kong, where both deceased-donor liver transplants (DDLTs) and living-donor liver transplants (LDLTs) are performed. All patients waitlisted for transplant are entered into a central transplant registry, and cadaveric donor liver grafts are allocated based on the Model for End-stage Liver Disease score, with adjustment for HCC status. However, cadaveric liver donation is uncommon in Hong Kong, possibly due to societal and cultural reasons.28 In the past decade, the annual number of liver donations from deceased donors has remained less than 40.29

To tackle the scarcity of deceased donor liver grafts, LDLT using extended right lobe grafts for adults has been pioneered in Hong Kong since 1996,30 and the technique has gained popularity in Asia. From 1996 to 2018, over 1,400 LDLTs have been performed in Hong Kong, with 16.5% of LDLTs performed for HCC.31

A local prospective study has compared LDLT against hepatectomy for HCC, where LDLT demonstrated a 1.5-fold higher 10-year overall survival (83% vs. 56% respectively) with 2-fold higher 10-year disease-free survival (81% vs. 40% respectively).32 Local data has also reported comparable 5-year oncological outcomes and survival when comparing LDLT and DDLT for HCC.33 LDLT is the predominant type of transplant surgery in Hong Kong, and it is an ideal HCC treatment in our locality.

All the recommended bridging therapy options, including
local ablation (RFA or HIFU), TACE, TARE and SBRT are performed in Hong Kong.\(^{34}\) In particular, TACE and SBRT are the most commonly performed. A recent local study demonstrated bridging SBRT was associated with a significantly higher 1-year tumor control when compared with TACE or HIFU, supporting SBRT as the top choice for bridging.\(^{35}\)

**CURATIVE TREATMENT – POST-TREATMENT SURVEILLANCE AND MANAGEMENT**

**Clinical guidelines**

No local guidelines in Hong Kong have covered post-treatment surveillance and management. Nonetheless, the US National Comprehensive Cancer Network recommendations are frequently referenced, where patients receive CT/ MR scans plus serum AFP testing every 3–6 months for 2 years after treatment, followed by surveillance at 6–12 month intervals thereafter.\(^{36}\)

**Real-life practice**

Surveillance after curative HCC therapy is overseen by surgeons in Hong Kong.\(^ {37,38}\) Close surveillance by CT/MR with AFP is generally adhered to, enabling early detection and management of recurrence. The use of dual-tracer PET-CT as surveillance has also been explored in post-transplant patients in a local study. Notably, dual-tracer PET-CT had increased sensitivity of recurrence detection by 12%, and led to change in management in one-third of post-transplant patients.\(^ {38}\)

As the majority of HCC in Hong Kong are CHB-related, antivirals are routinely used to reduce the recurrence risk after curative therapy for HCC. Despite close surveillance and antiviral therapy, local data has shown HCC recurrence rates of over 70% in hepatectomy and over 20% in liver transplant respectively, which are compatible with international data.\(^ {37,38}\) In patients with recurrence, treatment protocols are generally similar to that in patients with newly diagnosed HCC.

**NON-CURATIVE TREATMENT – LOCOREGIONAL THERAPY**

**Clinical guidelines**

TACE is recommended in unresectable HCC with no vascular invasion or extrahepatic spread and with satisfactory liver function. While patients with no vascular invasion are ideal candidates, segmental portal vein thrombosis and venous invasion are not absolute contraindications. TACE with drug-eluting beads (DEB-TACE) is a possible alternative if conventional TACE fails to achieve a tumor response.\(^5\)

Besides TACE, SBRT and TARE are other options for locoregional therapy for Child-Pugh A patients, especially for larger tumors (>5 cm). In particular, the versatility of SBRT is highlighted in that SBRT may be used for bridging to transplant, for downstaging HCC for resection, and to be used in combination with TACE.\(^5\)

**Real-life practice**

TACE is the first-line therapy for unresectable intermediate stage or locally-advanced HCC in Hong Kong. DEB-TACE is also available in some tertiary centers. A shared-care approach between surgeons and oncologists enable smooth transitioning of TACE-refractory patients to systemic therapy.\(^5\)

The use of SBRT for unresectable HCC, either alone or in combination with TACE, is increasing in Hong Kong.\(^ {39}\) Local experience has demonstrated that SBRT+TACE, when compared with TACE alone, led to significantly improved radiological disease control (98.0% vs. 56.7%), 1-year overall survival (67.2% vs. 36.5%), and 3-year overall survival (43.9% vs. 13.3%).\(^ {40}\) TARE is less frequently performed than TACE in Hong Kong. Limited local data has shown that TARE is safe and has similar 1-year survival when compared with TACE.\(^ {41,42}\)

**NON-CURATIVE TREATMENT – SYSTEMIC THERAPY**

**Clinical guidelines**

Systemic therapy is the last therapeutic option in patients with metastatic disease or in advanced HCC patients who
have failed locoregional therapy.

In the 2022 local guidelines, the combination of Atezolizumab+Bevacizumab is recommended as first-line systemic treatment, although this recommendation is restricted to patients with good liver function (Child-Pugh A). Nivolumab monotherapy is an alternative first-line systemic treatment for Child-Pugh B patients, and may be considered for patients with poorly-controlled hypertension or high bleeding risks. Tyrosine kinase inhibitor (TKIs) (Lenvatinib or Sorafenib) are also suitable as first-line treatment if the patient prefers oral treatment or have contraindications for immunotherapy.5,6

For patients who progress after first-line systemic therapy, second-line targeted therapy including Regorafenib, Cabozantinib or Ramucirumab are suitable options.5,6 Immunotherapy may be considered as second-line treatment for patients who failed first-line TKIs.9

Real-life practice

A range of international drug trials are ongoing in the academic centers in Hong Kong, and advanced HCC patients may be able to receive novel agents in drug trials.43,44 Outside of the context of clinical trials, the whole range of guideline-recommended targeted therapy and immunotherapy options are available locally. Nonetheless, treatment protocols are usually individualized due to patient preferences and tolerability. In Hong Kong, the use of targeted therapy and immunotherapy as approved therapies have to be paid out-of-pocket by patients, although subsidies may be available for some options from the government and non-government organizations. Cost concern is hence another important factor that impact drug choices for our HCC patients.

In recent years, the use of immunotherapy (Nivolumab monotherapy or Nivolumab+Ipilimumab) or immunotherapy combined with anti-angiogenic agents (e.g., Atezolizumab+Bevacizumab) as first-line systemic therapy is increasingly advocated in our locality due to data from the landmark IMbrave150, CheckMate459 and Checkmate040 trials respectively.45-47 Bleeding risks and cost are potential reasons in favour of using Nivolumab-containing regimens over Atezolizumab+Bevacizumab.

Poor liver reserve in some patients preclude them from receiving immunotherapy, and patients’ preferences for oral drugs are also frequently encountered in our locality.48 These factors lead to first-line TKIs to still have a prominent role for systemic therapy in Hong Kong. While Sorafenib and Lenvatinib are both recommended as first-line TKIs, Lenvatinib is generally preferred over Sorafenib in Hong Kong due to the superior disease control and overall response rate of Lenvatinib in the REFLECT trial.49

As per the guidelines, second-line use of immunotherapy or targeted therapy is practiced in Hong Kong.48 However when patients progress beyond second-line treatment, no clear instructions are available in the local guidelines, and treatment is based on expert-opinion. Exciting yet limited data has emerged in Hong Kong for treatment beyond second-line. For example, the combination of Nivolumab/Pembrolizumab+Ipilimumab has been used in patients who have failed prior immune checkpoint inhibitor therapy, where 1-year overall survival of 42.4% was achieved, with 12.0% of patients achieving complete response.17 The novel combination of Cabozantinib (TKI) with immunotherapy has also been reported in 15 patients, achieving a promising 1-year survival of 71.5%.50

DISCUSSION

In the past few decades, researchers from Hong Kong have produced high-impact studies that have shifted the paradigm of HCC management. With support from local data, multidisciplinary teams of practicing clinicians have developed local HCC guidelines that are up-to-date and practical.

In Hong Kong, deviation of real-life practice from HCC guidelines may represent efforts to improve HCC care based on novel data and expert experience. For example, the first-line use of dual-tracer PET-CT and the “off-label” use of immunotherapy for treatment-resistant HCC are exciting developments that may be increasingly adopted and may be incorporated in future versions of the guidelines. Real-life practice may also vary due to resource constraints and availability of expertise. Nonetheless, Hong Kong is unique in its relatively small geographical size (around 1,100 km² only), and referral of patients to specialized tertiary centers is easily achievable and frequently performed.51

Despite the strengths of the HCC guidelines in Hong Kong, several areas can be improved on. First, the current guidelines have not provided recommendations on HCC diagnosis. As Hong Kong has an increasing availability of advanced im-
aging services,\textsuperscript{9,14,20} local guidelines on diagnostic pathways will be beneficial to enable earlier and more accurate diagnosis of HCC.

Second, post-treatment surveillance and risk reduction are important areas that should be discussed in the guidelines as well. The use of antivirals for CHB is routinely used to reduce HCC recurrence in Hong Kong. Nonetheless, local data has highlighted other risk factors for HCC in CHB patients such as fibrotic burden and metabolic risk factors.\textsuperscript{52-54} In fact, a local study on a mixed cohort of HCC patients highlighted hepatic steatosis as a key risk factor for HCC recurrence.\textsuperscript{50} Guidelines are warranted to guide the post-treatment follow-up plan for HCC, with potential need to include metabolic screening as a measure to reduce recurrence.

Finally, supportive care for HCC is an area that has not been covered in the local guidelines. Palliative care in hepatology is a topic that is gaining increasing attention in recent years.\textsuperscript{56} In particular, palliative care in HCC is unique given the uncertain disease course, risk of rapid progression, and the need to care for both malignancy-related and cirrhosis-related symptoms.\textsuperscript{56} Guidance for symptomatic care may improve the quality-of-life for advanced HCC patients that have failed other therapies.

Overall, the HCC guidelines in Hong Kong are practical and up-to-date. The guidelines are tailored based on our unique population and local expertise, and are able to assist our daily practice. They are mostly adhered to in the current clinical practice in Hong Kong. Nonetheless, the field of HCC management is rapidly evolving, and regular updates of our guidelines will be required to incorporate the novel data that is consistently emerging from local and international studies.

Authors’ contribution

RWHH was involved in data interpretation and drafting of the manuscript. LYM, TTC, VHFL and WKS were involved in critical revision of the manuscript. MFY was involved in study concept, critical revision of the manuscript, and overall study supervision. All authors have seen and approved the final version of the manuscript.

Conflicts of Interest

MF Yuen is an advisory board member and/or received research funding from AbbVie, Arbutus Biopharma, Assembly Biosciences, Bristol Myer Squibb, Dicerna Pharmaceuticals, GlaxoSmithKline, Gilead Sciences, Janssen, Merck Sharp and Dohme, Clear B Therapeutics, Springbank Pharmaceuticals, and received research funding from Arrowhead Pharmaceuticals, Fujirebio Incorporation and Sysmex Corporation. WK Seto received speaker’s fees from AstraZeneca and Mylan, is an advisory board member of CSL Behring, is an advisory board member and received speaker’s fees from AbbVie, and is an advisory board member, received speaker’s fees and researching funding from Gilead Sciences. The remaining authors have no conflict of interests.

REFERENCES


http://www.e-cmh.org


Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Taiwan perspective

Tung-Hung Su1,2, Chih-Horng Wu2,3, Tsung-Hao Liu4,5, Cheng-Maw Ho2,6, and Chun-Jen Liu1,2,7

1Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei; 2Hepatitis Research Center, National Taiwan University Hospital, Taipei; 3Department of Medical Imaging, National Taiwan University Hospital, Taipei; 4Department of Oncology, National Taiwan University Hospital, Taipei; 5Graduate Institute of Oncology, National Taiwan University University College of Medicine, Taipei; 6Department of Surgery, National Taiwan University Hospital, Taipei; 7Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

Hepatocellular carcinoma (HCC) is the fourth most common cancer and the second leading cause of cancer-related death in Taiwan. The Taiwan Liver Cancer Association and the Gastroenterological Society of Taiwan developed and updated the guidelines for HCC management in 2020. In clinical practice, we follow these guidelines and the reimbursement policy of the government. In Taiwan, abdominal ultrasonography, alpha-fetoprotein, and protein induced by vitamin K absence or antagonist-II (PIVKA-II) tests are performed for HCC surveillance every 6 months or every 3 months for high-risk patients. Dynamic computed tomography, magnetic resonance imaging, and contrast-enhanced ultrasound have been recommended for HCC surveillance in extremely high-risk patients or those with poor ultrasonographic visualization results. HCC is usually diagnosed through dynamic imaging, and pathological diagnosis is recommended. Staging of HCC is based on a modified version of the Barcelona Clinic Liver Cancer (BCLC) system, and the HCC management guidelines in Taiwan actively promote curative treatments including surgery and locoregional therapy for BCLC stage B or C patients. Transarterial chemoembolization (TACE), drug-eluting bead TACE, transarterial radioembolization, and hepatic artery infusion chemotherapy may be administered for patients with BCLC stage B or C HCC. Sorafenib and lenvatinib are reimbursed as systemic therapies, and regorafenib and ramucirumab may be reimbursed in cases of sorafenib failure. First-line atezolizumab with bevacizumab is not yet reimbursed but may be administered in clinical practice. Systemic therapy and external beam radiation therapy may be used in specific patients. Early switching to systemic therapy in TACE-refractory patients is a recent paradigm shift in HCC management. (Clin Mol Hepatol 2023;29:230-241)

Keywords: Liver cancer; Surveillance; Barcelona clinic liver cancer; Surgery; Systemic therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth most common cancer in Taiwan. The Taiwan Cancer Registry reported 11,272 new HCC cases in 2019, with a crude incidence rate of 47.76 per 100,000 person-years. Moreover, 7,881 HCC mortalities occurred, and the crude mortality rate was 33.39 per 100,000 person-years; thus, HCC constitutes the second leading cause of cancer-related mortality in Taiwan. HCC cases in Taiwan are mostly attributable to hepatitis B virus (HBV) in-
fection (47%), followed by that of hepatitis C virus (HCV) (33%). Active viral replication is the primary mechanism of hepatocarcinogenesis.2

Taiwan was the first country to launch nationwide HBV vaccination in 1984;3 this decreased the HBV carriage rate and reduced the risk of developing HCC (as primary prevention).4 Antiviral therapy reduces the risk of HCC caused by both HBV5,6 and HCV (as secondary prevention).7 Antiviral therapy reduces the incidence of recurrence of HBV- and HCV-related HCC after curative therapies (as tertiary prevention).8,9 The National Health Insurance (NHI) program in Taiwan has reimbursed anti-HBV and anti-HCV therapy since 2003, which has effectively reduced HCC incidence and mortality attributable to viral hepatitis.10 The National Hepatitis C Program Office launched a step-wise intervention to eradicate chronic hepatitis C. Since 2017, Taiwan has fully reimbursed prescriptions of direct antiviral agents (DAAs)—initially for patients with cirrhosis and later for patients regardless of fibrosis status. By June 30, 2022, more than 130,000 patients with chronic hepatitis C had been treated with DAA.

Overall, the incidence of HBV- and HCV-related HCC is decreasing, whereas the incidence of non-HBV- or non-HCV-related HCC is increasing. As in other parts of the world, nonalcoholic steatohepatitis caused by westernization of lifestyle practices or alcoholism is an emerging etiology of HCC. HCC caused by primary biliary cholangitis, autoimmune hepatitis, or aflatoxin is not common in Taiwan. The Taiwan Liver Cancer Association (TLCA) and the Gastroenterological Society of Taiwan (GEST) proposed a management consensus for HCC in 2016,1 which was updated in 2020.11

## HCC SURVEILLANCE

### Clinical guidelines

TLCA guidelines specify that patients with chronic hepatitis B or C and cirrhosis are at high risk of HCC and should enroll in a surveillance program for HCC that provides opportunities for curative treatment and improves overall survival.12 Surveillance should be performed using abdominal ultrasonography and alpha-fetoprotein tests (both are covered by the NHI program) at 6-month intervals (with a range of 3–12 months).1 Dynamic computed tomography (CT), magnetic resonance imaging (MRI), or gadolinium ethoxybenzyl-diethylenetriaminepentaacetic acid (Gd-EOB-DTPA)-enhanced MRI (EOB-MRI) may be recommended every 6 to 12 months for extremely high-risk patients and for patients with difficulty in ultrasound imaging of the liver because of liver atrophy, severe obesity, or postoperative deformity.13 Kupffer-phase contrast-enhanced ultrasound (CEUS) may also be recommended as a first-line screening tool for HCC in patients with renal dysfunction and liver cirrhosis (Table 1).1,13,14

### Real-world practice

A major discrepancy exists between the execution of the guidelines and real-world practice due to poor patient adherence to surveillance recommendations. According to the NHI claim database, among 685,000 patients with a primary diagnosis of hepatitis or cirrhosis in 2008, only 13% received ultrasound and alanine aminotransferase examinations every 6 months. To facilitate regular surveillance of HCC, the National Health Insurance Administration of Taiwan introduced a medical care improvement plan in 2000 for patients with chronic hepatitis B or C. This patient-centered program is intended to motivate physicians to perform regular ultrasonography for HCC surveillance every 6 months as recommended by the current guidelines and to encourage HCC identification in the early stage through additional reimbursement to institutions. Examination of protein induced by vitamin K absence or antagonist-II (PIVKA-II) every 6 months has been reimbursed by NHI since 2020 for patients with cirrhosis and those receiving curative therapy for HCC. However, EOB-MRI and CEUS are not reimbursed by NHI.

### Abbreviations:

HCC, hepatocellular carcinoma; PIVKA-II, protein induced by vitamin K absence or antagonist-II; BCLC, Barcelona Clinic Liver Cancer; TACE, transarterial chemoembolization; HBV, hepatitis B virus; HCV, hepatitis C virus; NHI, National Health Insurance; DAA, direct antiviral agent; TLCA, Taiwan Liver Cancer Association; GEST, Gastroenterological Society of Taiwan; CT, computed tomography; MRI, magnetic resonance imaging; EOB-MRI, Gadolinium ethoxybenzyl-diethylenetriaminepentaacetic acid enhanced MRI; CEUS, contrast-enhanced ultrasound; APASL, Asian Pacific Association for the Study of the Liver; AASLD, American Association for the Study of Liver Disease; Li-RADS, Liver Imaging Reporting and Data System; EASL, European Association for the Study of the Liver; HAIC, hepatic arterial infusion chemotherapy; ALPPLS, Associating Liver Partition and Portal vein Ligation for Staged hepatectomy; RFA, radiofrequency ablation; DEB, drug-eluting bead; EBRT, external beam radiation therapy.
**DIAGNOSIS**

**Radiological diagnosis**

In Taiwan, HCC can be diagnosed noninvasively through dynamic contrast-enhanced CT or MRI if a ≥1.0 cm lesion is identified through ultrasound during surveillance.\(^{11}\) Furthermore, guidelines in Asian countries, including those of the Japan Society of Hepatology and the Asian Pacific Association for the Study of the Liver (APASL), recommend EOB-MRI as the first-line diagnostic tool because it is more sensitive than dynamic CT for diagnosing HCC.\(^{15,16}\) Additionally, EOB-MRI performed after dynamic CT in patients with early-stage HCC can detect additional small nodules, increase the accuracy of cancer staging, and improve outcomes after curative treatment.\(^{17,18}\) However, the cost of EOB-MRI is not covered by the NHI program in Taiwan even though it is categorized as both a first- and second-line imaging diagnostic tool.\(^{10}\)

The guidelines of the American Association for the Study of Liver Disease (AASLD) standardize the terminology for interpreting imaging features indicating the presence of HCC; the American College of Radiology released the Liver Imaging Reporting and Data System (LI-RADS) in 2011.\(^ {19}\) LI-RADS describes the following categorization: LI-RADS 1 (LR-1, definitely benign), LI-RADS 2 (LR-2, probably benign), LI-RADS 3 (LR-3, intermediate probability), LI-RADS 4 (LR-4, probably HCC), LI-RADS 5 (LR-5, definitely HCC), LI-RADS M (LR-M, malignant but not HCC specific), and LI-RADS TIV (LR-TIV, tumor in vein) based on the likelihood of HCC, non-HCC malignancy, and venous tumors. The Taiwan Society of Interventional Radiology has introduced the use of the LI-RADS in clinical practice for liver tumor diagnosis. However, no study has compared the diagnostic performance and clinical value of LI-RADS v2018.

**Pathology diagnosis**

**Clinical guideline**

The TLCA guidelines support the clinical diagnosis of HCC in high-risk patients with liver nodules of size >1 cm with a background of cirrhosis or chronic hepatitis B or C. This recommendation is in concordance with AASLD, European Association for the Study of the Liver (EASL), APASL, and other guidelines from major academic organizations.\(^ {11,16,20-23}\) Histological proof is required when the clinical diagnostic criteria of HCC are not satisfied or the diagnosis of HCC is not of high certainty. TLCA guidelines promote an active biopsy strategy and specify the requirement of histological proof for liver tumors. Although histological subtypes and gene signatures have not yet become key informations before HCC treatment, clinical trials and research are dependent on the availability of HCC tissues. Risks associated with biopsy, including bleeding and needle track tumor spreading tumor spreading,\(^ {24}\) although small, should be considered when contemplating tumor biopsy.

**Real-world practice**

With the recommendation of histological proof in the TLCA guidelines and the increasing number of immunotherapy combination trials in HCC, physicians in Taiwan have adopted

---

**Table 1. Comparison of HCC surveillance programs between international and Taiwan guidelines and real-world practice**

<table>
<thead>
<tr>
<th>HCC surveillance</th>
<th>International/other guidelines(^ {13,14,16,20,21})</th>
<th>Taiwan guideline(^ {1,11})</th>
<th>Real-world practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Alpha-fetoprotein</td>
<td>No: EASL</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Optional: AASLD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes: APASL, NCCN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIVKA-II</td>
<td>Yes: JSH</td>
<td>No</td>
<td>Yes (cirrhosis/HCC curative therapy)</td>
</tr>
<tr>
<td>CT/MRI/CEUS</td>
<td>CT/MRI in extremely high risk patients (JSH)</td>
<td>Yes in extremely high risk patients (6–12 months)</td>
<td>Yes, but not reimbursed by National Health Insurance</td>
</tr>
<tr>
<td>Interval</td>
<td>6 months</td>
<td>6 months (3–12 months)</td>
<td>3–12 months</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; PIVKA-II, protein induced by vitamin K absence or antagonist-II; EASL, European Association for the Study of the Liver; AASLD, American Association for the Study of Liver Diseases; APASL, Asian Pacific Association for the Study of the Liver; NCCN, National Comprehensive Cancer Network; JSH, Japan Society of Hepatology; CT, computed tomography; MRI, magnetic resonance imaging; CEUS, contrast-enhanced ultrasound.
a more aggressive attitude toward active tumor biopsy, particularly in medical centers with clinical trial participation. In 2019, 48.2% of HCC diagnoses were supported by pathology or cytology, which contrasts with the rate <40% being supported by pathology or cytology before 2000 according to the Taiwan Cancer Registry report.

STAGING

Clinical practice guidelines

The Barcelona Clinic Liver Cancer (BCLC) staging system stratifies patients with HCC into very early, early, intermediate, and advanced stages, with 5-year survival rates of 40–70%, 14–45%, 6–14%, and 10%, respectively, and the terminal stage, for patients with tumors beyond the transplantation threshold. Because of improvements in the HCC surveillance program, the proportion of patients with HCC diagnosed in the early stage has increased from 5–10% to 40–60%, leading to more patients eligible for curative treatments.

Accurate identification of the tumor (T) stage is crucial for extending disease-free survival after curative treatment because tumor size, tumor number, and microvascular invasion are significant predictors of survival. These tumor characteristics can be examined through preoperative imaging such as liver dynamic CT and MRI. The strengths of MRI include low operator dependence, no radiation exposure, and ability to analyze the whole liver parenchyma. Furthermore, EOB-MRI has detected more HCCs than dynamic CT in 16.4% of patients receiving concurrent EOB-MRI. Studies have suggested that higher numbers of HCCs necessitate a change in BCLC staging system, TNM staging, and treatment strategy. However, liver MRI usually do not visualize lung clearly, which may be the most common area of metastasis in HCC. Therefore, additional liver MRI is suggested in patients with very-early to early-stage HCC, and whole-body CT is recommended for patients with intermediate to advanced HCC.

Real-world practice

TLCA guidelines recognize the BCLC staging system as the most common in Taiwan in terms of prognostic prediction. The BCLC staging system used in Taiwan has two modifications from the original system. One is that, since 2002, a single tumor of size >5 cm has been classified as BCLC stage B; the other is that a patient with Eastern Cooperative Oncology Group (ECOG) performance status 1 can still be classified as stage 0, stage A, or stage B according to the tumor burden (Table 2). These differences must be noted when comparing the prognosis of patients with HCC of various BCLC stages in Taiwan and with those in other countries. Other staging systems, including the HKLC staging system, CLIP score, Tokyo score, Japan Integrated Staging score, and TNM system, also provide meaningful prognosis predictions. Another key goal of staging systems is to inform treatment selection. Although the BCLC staging system is the most used in Tai-

<table>
<thead>
<tr>
<th>Stage</th>
<th>Tumor burden</th>
<th>Liver function</th>
<th>Performance status</th>
<th>Tumor burden</th>
<th>Liver function</th>
<th>Performance status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Single ≤2 cm</td>
<td>Preserved liver function</td>
<td>0</td>
<td>Single ≤2 cm</td>
<td>Child-Pugh A</td>
<td>0–1</td>
</tr>
<tr>
<td>A</td>
<td>Single or ≤3 nodules each ≤3 cm</td>
<td>Preserved liver function</td>
<td>0</td>
<td>Single ≤5 cm or ≤3 nodules each ≤3 cm</td>
<td>Child-Pugh A-B</td>
<td>0–1</td>
</tr>
<tr>
<td>B</td>
<td>Multinodular</td>
<td>Preserved liver function</td>
<td>0</td>
<td>Single &gt;5 cm or Multinodular</td>
<td>Child-Pugh A-B</td>
<td>0–1</td>
</tr>
<tr>
<td>C</td>
<td>Portal invasion, N1, M1</td>
<td>Preserved liver function</td>
<td>1–2</td>
<td>Portal invasion, N1, M1</td>
<td>Child-Pugh A-B</td>
<td>0–2</td>
</tr>
<tr>
<td>D</td>
<td>Any</td>
<td>End-stage liver function</td>
<td>3–4</td>
<td>Any</td>
<td>Child-Pugh C</td>
<td>3–4</td>
</tr>
</tbody>
</table>

BCLC, Barcelona Clinic Liver Cancer.
A shortage of transplantable organs from deceased individuals in Taiwan necessitates the development of living-donor liver transplantation. The Milan criteria and UCSF criteria for liver transplantation for eligible patients with HCC are practiced. Salvage transplantation using liver resection as the primary treatment for patients, followed by transplantation in the event of HCC recurrence or liver failure does not increase the risk of recurrence or similar long-term outcomes compared with primary liver transplantation. The overall transplantable pool of patients after resection has not decreased. Downstaging and bridging treatment should be offered to all patients to avoid waitlist dropout. The estimated wait time for transplantation is more than 6 months. Less strict criteria and incorporation of biological markers are being used in patient selection worldwide, and their long-term effects in Taiwan require investigation.

Radiofrequency ablation, transarterial chemoembolization, and radioembolization

Radiofrequency ablation (RFA) is a safe and effective curative therapy for patients with very early or early stage HCC who are unsuitable for surgery. Furthermore, the percutaneous approach for RFA has the advantages of lower morbidity and a shorter length of hospital stay because of its minimal invasiveness. Because of its safety, simplicity, and cost, ultrasound is vital in guiding needle insertion and monitoring the ablation effect during RFA. Moreover, in ultrasound-guided RFA, many strategies (e.g., artificial ascites or pleural effusion creation, real-time ultrasound-CT/MRI fusion imaging, and CEUS) can be used to decrease the incidence of complications and increase the rate of complete ablation. However, because of the limitations in the ultrasound window and resolution, ultrasound-guided RFA in tumors with difficult locations and poor visibility is associated with a higher local recurrence rate.

In contrast, CT-guided RFA presents no limitation to the depth and field of view. However, one study has reported comparable efficacy and complications between ultrasound- and CT-guided RFA for HCC. Additionally, the combination of transarterial chemoembolization (TACE) and RFA may lead to longer hospital stays and increased patient discomfort. Wu et al. reported that CT-guided RFA after intra-arterial iodized oil injection may achieve more prolonged recurrence-free survival than ultrasound guidance, and that CT-guided
RFA is more suitable in this clinical context.

TACE has served as a first-line treatment for intermediate to advanced HCC for over a decade. Other intra-arterial therapies, such as drug-eluting beads (DEBs), transarterial radioembolization, and HAIC, are available in Taiwan. These techniques provide interventional radiologists in Taiwan with more options for unresectable HCC treatment. Although the NHI program does not reimburse DEB-TACE or transarterial radioembolization, a consensus exists in Taiwan on the DEB-TACE recommendation, and physicians have experience attending randomized controlled trials of transarterial radioembolization. HAIC is also recommended for patients with portal vein thrombosis, but no consensus or large-scale randomized controlled trial exists. Practice guidelines recommend DEB-TACE, transarterial radioembolization, and HAIC for patients with multiple tumors or vascular invasion.

SYSTEMIC THERAPY

Clinical guidelines

TLCA guidelines recommends sorafenib and lenvatinib therapy for treatment-naive patients with Child–Pugh A liver function, Eastern Cooperative Oncology Group (ECOG) performance status ≤2, and HCC that is unresectable and not amenable to locoregional therapy or is refractory to TACE. Atezolizumab and bevacizumab combination therapy can be used for treating patients with unresectable HCC who have not received prior systemic therapy and do not have a high risk of upper gastrointestinal bleeding. Sorafenib or nivolumab immunotherapy may be considered for selected patients with Child Pugh class B liver function whose tumors are unresectable and not amenable to locoregional therapy, but the evidence remains insufficient.

In cases of disease progression after sorafenib, additional treatment with regorafenib, cabozantinib, and ramucirumab (when Alpha-fetoprotein ≥400 ng/mL) extend the survival of patients with HCC and Child Pugh class A liver function, whose tumors are unresectable and not amenable to locoregional therapy. Nivolumab with or without ipilimumab or pembrolizumab can be considered for patients who are intolerant to or have progressed when treated with approved tyrosine kinase inhibitors (Table 3).

A paradigm shift in adopting systemic therapy in BCLC stage B HCC has occurred. TLCA guidelines and clinical studies suggest that targeted therapy combined with TACE can be considered in highly selected patients with unresectable BCLC stage B HCC with Child Pugh class A and ECOG performance status 0-1.

Real-world practice

Because of economic factors, real-world practice mainly depends on the reimbursement criteria of the NHI program. The NHI program has reimbursed sorafenib and lenvatinib as first-line therapy since 2012 and 2020, respectively. To receive sorafenib and lenvatinib treatment, HCC must exhibit extrahepatic spread, major vascular invasion (Vp 2-4), or be refractory to TACE, which is defined as failure to respond to more than 3 TACE sessions within 12 months. Lee et al. investigated 22 and 44 BCLC stage C patients who received first-line lenvatinib and sorafenib, respectively. The objective response rate (ORR; 36.4% vs. 11.4%, P=0.023) and disease control rate (DCR) (81.9% vs. 56.9%, P=0.039) were higher in the lenvatinib group than in the sorafenib group, but patients had a similar overall survival of approximately 9 months. No first-line immunotherapy is currently reimbursed by the NHI program in Taiwan. In clinical practice, patients receive treatment regimens such as atezolizumab with bevacizumab or lenvatinib with pembrolizumab based on shared decision-making between physician and patient. Shao et al. evaluated 40 participants from Taiwan in the IMbrave 150 and the GO30140 trials. The ORR was 37.5%, including 3 (7.5%) complete responses, and the median duration of response was 21.4 months (95% confidence interval, 16.6-not reached), which was consistent with the findings for the global intent-to-treat populations. Wu et al. evaluated 71 patients who received lenvatinib plus pembrolizumab for unresectable HCC and reported an ORR of 34.1% in the first-line setting and of 18.5% for systemic therapy-experienced cases. Regorafenib and ramucirumab are reimbursed by the NHI program as second-line therapy in cases of failed first-line sorafenib administration. Nivolumab monotherapy had previously been reimbursed after failure of sorafenib; however, since April 2020, it is no longer reimbursed for new cases.
<table>
<thead>
<tr>
<th>Systemic treatments</th>
<th>International guideline</th>
<th>Taiwan guideline</th>
<th>Real-world practice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First line</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target therapy</td>
<td>Sorafenib, lenvatinib</td>
<td>Sorafenib, lenvatinib</td>
<td>Sorafenib, lenvatinib*</td>
</tr>
<tr>
<td>Immunotherapy or immunotherapy</td>
<td>Atezolizumab+bevacizumab</td>
<td>Atezolizumab+bevacizumab</td>
<td>Atezolizumab+bevacizumab, lenvatinib+ pembrolizumab, bevacizumab+anti-PD-1 monodonal antibody†</td>
</tr>
<tr>
<td>combinations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytotoxic chemotherapy</td>
<td>No</td>
<td>Selected patients (regimen not specified)</td>
<td>FOLFOX, Cisplatin+infusional 5-FU, doxorubicin</td>
</tr>
<tr>
<td><strong>Second line</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target therapy</td>
<td>Regorafenib, cabozantinib, ramucirumab</td>
<td>Regorafenib, cabozantinib, ramucirumab</td>
<td>Regorafenib, Ramucirumab</td>
</tr>
<tr>
<td>Immunotherapy or immunotherapy</td>
<td>Nivolumab+ipilimumab, pembrolizumab</td>
<td>Nivolumab+ipilimumab, pembrolizumab</td>
<td>Nivolumab+ipilimumab, pembrolizumab, multikinase inhibitor+anti-PD-1/PD-L1 monodonal antibody†, bevacizumab+anti-PD-1 monoconal antibody‡</td>
</tr>
<tr>
<td>combinations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Systemic agents that are currently reimbursed by the National Health Insurance in Taiwan in year 2022.

†Low dose bevacizumab in combination with anti-PD-1 monoclonal antibody (e.g., nivolumab or pembrolizumab) as an alternative to atezolizumab plus bevacizumab for a lower-financial burden off-label usage.

‡Multikinase inhibitors (lenvatinib, regorafenib, or sorafenib) in combination with anti-PD-1 monoclonal antibody (e.g., nivolumab or pembrolizumab) as an alternative off-label agent.
Clinical trial participation is highly encouraged in Taiwan and is supported by the government (https://www.taiwan-clinicaltrials.tw/). Many landmark trials are spearheaded by investigators in Taiwan, including those for sorafenib (A-P study), lenvatinib (REFLECT study), nivolumab (CheckMate 040 study), and the atezolizumab–bevacizumab combination (IMbrave 150, GO30140). Taiwan has an outstanding health-care system with 23 medical centers and up to 99.96% population coverage by the NHI program, which provides an excellent environment for clinical trial implementation. Medical centers in Taiwan actively recruit patients to clinical trials involving early-, intermediate-, and advanced-stage HCC with the belief that all suitable patients should be offered the opportunity to be considered for participation.

TLCA guidelines endorse the use of chemotherapy for HCC as both systemic and locoregional therapy. Systemic chemotherapy commonly demonstrates 5–10% response rates in patients with HCC with acceptable performance status and liver reserve. However, HAIC is a form of locoregional therapy with a response rate up to 30% and is valuable for intrahepatic tumor control. A phase III study demonstrated the survival benefit of combining sorafenib with FOLFOX compared with sorafenib alone in patients with portal vein tumor thrombosis (PVTT). This response rate of sorafenib with FOLFOX was 40.8%, which may be of great value in patients with large intrahepatic tumor burden or PVTT. Although phase III studies of systemic chemotherapy (FOLFOX, PIAF, or doxorubicin) have not demonstrated a clear survival benefit for patients with advanced HCC and only one clinical trial reported survival benefit of adding sorafenib to HAIC-FOLF-OX, both systemic therapy and HAIC remain in the armamentarium of the physician treating HCC in Taiwan because of the high response rate for intrahepatic tumor control and reimbursement by the NHI program.

TLCA guidelines support the administration of external beam radiation therapy (EBRT), including photon and proton therapy, for various stages of HCC. For BCLC stage A, EBRT can be considered when HCC is inaccessible to ablation or is unresectable, as a bridge therapy before liver transplantation, or when the patient refuses standard treatment. For BCLC stage B, EBRT can be considered in cases where HCC is inaccessible or unsuitable for TACE or is refractory to TACE, as a bridge to liver transplantation, or when localized tumor with symptoms or a threat to liver reserve is present. For BCLC stage C, EBRT can be considered in patients with portal vein tumor thrombus, in those with HCC unsuitable or refractory to TACE, or in those with a localized tumor with symptoms or a threat to liver reserve. For BCLC stage D, EBRT can be considered for symptomatic metastasis or for oligometastases as palliation. In real practice, EBRT in addition to standard therapy is not uncommon and is favored by a subset of patients and physicians in Taiwan.

DISCUSSION

Because of the high disease burden of HCC and the high-quality medical care reimbursed by the NHI program in Taiwan, TLCA guidelines devote considerable attention to preventing the development, pursuing the early diagnosis, and improving the overall survival of HCC. Compared with the BCLC guidelines, TLCA guidelines advocate a more aggressive attitude toward curative treatment (e.g., surgical resection). Whenever possible, surgical intervention is considered first for managing HCC. Liver transplantation is not yet widely applied to patients with HCC, even in the setting of living donor predominance.

The introduction of systemic therapy has greatly contributed to the management strategies available for intermediate- and advanced-stage HCC. Physicians in Taiwan typically attempt to downstage HCC for curative therapy. The BCLC-guided treatment is advanced or modified according to the therapeutic effectiveness of locoregional or systemic therapy in each scenario. For intermediate-stage HCC, systemic therapy may be neoadjuvant, early-switch therapy, adjuvant, or even initial therapy. However, the major limitation is lack of reimbursement by the NHI program in Taiwan. Currently, first-line immunotherapy is not reimbursed, which may reduce the overall treatment responses in advanced HCC.

HCC management is characterized by a constant struggle between treating the tumor and preserving residual liver function. Through a multidisciplinary team approach, application of antiviral therapy, and improvement of supportive care, liver reserve can be maintained after HCC management. In-depth, cross-professional communication between sur-
geons, hepatologists, oncologists, and interventional radiologists may provide the greatest benefits in caring for patients with HCC.

Randomized phase III trials may not provide optimal benefits for patients with HCC. Thus, in addition to randomized trials, high-quality real-world data and real-world evidence are required and will gradually play a greater role in drug approval. Considerable discrepancies exist between HCC guidelines and real-life practice. Academic organizations should recognize the inherent value of a multidisciplinary team approach in HCC treatment and endorse various modalities that may help patients with HCC.

**Authors’ contribution**

Tung-Hung Su: study concept and design, acquisition of data, analysis and interpretation of data, drafting the manuscript. Chih-Horng Wu: acquisition of data, analysis and interpretation of data, drafting the manuscript. Tsung-Hao Liu: acquisition of data, analysis and interpretation of data, drafting the manuscript. Cheng-Maw Ho: acquisition of data, analysis and interpretation of data, drafting the manuscript. Chun-Jen Liu: study concept and design, acquisition of data, analysis and interpretation of data, critical review and revise the manuscript, study supervision.

**Acknowledgements**

This work was supported by grants from the Ministry of Science and Technology, Taiwan (grant numbers MOST 109-2326-B-002 -012 -MY3, MOST 110-2326-B-400-004, MOST 110-2628-B-002-041), Ministry of Health and Welfare (MOHW111-TDU-B-221-014003), National Taiwan University Hospital (grant numbers 110-N01, 110-T20), and the Liver Disease Prevention & Treatment Research Foundation, Taiwan.

**Conflicts of Interest**

T.-H. S. received research grant from Gilead Sciences, served as a consultant for Gilead Sciences, and was on speaker’s bureaus for Abbvie, Bayer, Bristol-Myers Squibb, Gilead Sciences, Lilly, Merck Sharp and Dohme, Roche, and Takeda.

**REFERENCES**

2. Surveillance group; Diagnosis group; Staging group; Surgery group; Local ablation group; TACE/TARE/HAI group; Target therapy/systemic therapy group; Radiotherapy group; Prevention group; Drafting group. Management consensus guideline for hepatocellular carcinoma: 2016 updated by the Taiwan Liver Cancer Association and the Gastroenterological Society of Taiwan. J Formos Med Assoc 2018;117:381-403.
11. Shao YY, Wang SY, Lin SM; Diagnosis Group; Systemic Therapy Group. Management consensus guideline for hepatocellular carcinoma: 2020 update on surveillance, diagnosis, and systemic treatment by the Taiwan Liver Cancer Association and


2021;32:801-805.
Clinical practice guidelines and real-life practice in hepatocellular carcinoma: A Japanese perspective

Hironori Koga1,2, Hideki Iwamoto1,2, Hiroyuki Suzuki1,2, Shigeo Shimose1, Masahito Nakano1, and Takumi Kawaguchi1

1Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume; 2Liver Cancer Research Division, Research Center for Innovative Cancer Therapy, Kurume University, Kurume, Japan

Striking advances in systemic therapy for unresectable advanced hepatocellular carcinoma (HCC) have improved the average prognosis of patients with HCC. As a result, the guidelines for the treatment of HCC have changed significantly. However, various issues have emerged in clinical practice. First, there is no established biomarker that can predict response to systemic therapy. Second, there is no established treatment regimen after primary systemic therapy, including combined immunotherapy. Third, there is no established treatment regimen for intermediate-stage HCC. These points make the current guidelines ambiguous. In this review, we present the Japanese guidelines for the diagnosis and treatment of HCC based on the latest evidence; introduce various efforts mainly in Japanese real-life practice to update these guidelines; and present our perspectives on future guidelines. (Clin Mol Hepatol 2023;29:242-251)

Keywords: BCLC staging; Radiofrequency ablation; Transcatheter arterial chemoembolization; Molecular-targeted agents; Immune checkpoint inhibitors

INTRODUCTION

Primary liver cancer is the fifth most common cause of death in Japan1 and remains a serious disease in the national healthcare system. The number of new patients with hepatitis C virus (HCV)-related liver cancer, which previously accounted for 80% of all liver cancers in Japan, has continued to decline.2,3 Currently, it accounts for approximately 40% of all cases. However, prevalence of non-viral liver cancers is increasing. An efficient surveillance system for this type of liver cancer, occurring in association with metabolic disorders such as nonalcoholic fatty liver diseases,4 diabetes mellitus, and alcohol overdose, has not been developed. It is especially challenging when the liver cancer is already advanced at the time of diagnosis in patients with such metabolic disorders.

Recently, a paradigm change in the systemic treatment of advanced hepatocellular carcinoma (HCC) has occurred globally. In addition to multikinase inhibitors such as sorafenib and lenvatinib, combined immunotherapy such as atezolizumab plus bevacizumab is now widely used in clinical practice as a first-line systemic therapy, with real-world reports of improved prognosis.5,6 Furthermore, another combination therapy with immune checkpoint inhibitors (ICIs) will soon be covered by health insurance.7 However, issues such as the
establishment of biomarkers to predict response and how to proceed with conversion therapy combined with locoregional therapies remain unaddressed. In this article, we will review the current status of the clinical management of HCC in Japan, including recent findings and future trends based on the Guidelines for Liver Cancer Treatment 2021 published by the Japan Society of Hepatology (JSH).  

SURVEILLANCE AND DIAGNOSIS

Clinical practice guidelines overview

Patients with any of the following conditions, cirrhosis, chronic hepatitis B, or chronic hepatitis C, are considered to be at high risk for HCC, and those with cirrhosis type B (hepatitis B surface antigen-positive) and C (anti-HCV antibody-positive) are considered to be extremely high-risk groups for HCC. Ultrasonography (US) is considered a preferred surveillance modality with simultaneous measurements of alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), and the AFP-L3 fraction (a lectin-reactive fraction of AFP). US surveillance should be performed every six months in high-risk patients and every 3–4 months in extremely high-risk patients in Japan.

Dynamic computed tomography (CT) or dynamic magnetic resonance imaging (MRI), including gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MRI, can be combined with US surveillance in extremely high-risk patients and/or in patients whose livers are difficult to scan using US due to liver atrophy, severe obesity, and post-operative deformity. When nodular lesions are detected by US, CT/MRI is performed for differential diagnosis. Even when a tumor is not detected via US, dynamic CT/MRI should be performed every six months in high-risk patients and every 3–4 months in extremely high-risk patients in Japan.

Real-life practice

A major feature of the Japanese surveillance system is the use of the tumor markers AFP-L3 fraction and DCP. The assessment of these markers is covered under public health insurance. The addition of AFP-L3 assessment to US+AFP assessment is known to improve sensitivity in the diagnosis of HCC and is routinely used for surveillance extremely high-risk patients with chronic liver diseases. For such patients, simultaneous measurement of the tumor markers, AFP, AFP-L3, and DCP, is allowed under the health insurance system in Japan. Currently, its cost is 2,900 yen (approximately 22 dollars). Considering that the cost for independently measuring AFP, AFP-L3, and DCP is 1,010, 1,900, and 1,350 yen, respectively, amounting to 4,260 yen (approximately 32 dollars), it can be inferred that simultaneous measurement of the three markers helps to suppress over-measurement of tumor markers by medical institutions and to reduce expenditures from the insurance fund. Regarding MRI, most hepatologists in Japan prefer to use Gd-EOB-DTPA-enhanced MRI over conventional dynamic MRI with extracellular contrast agents. Recently, it has been suggested that Gd-EOB-DTPA-enhanced MRI may play an important role in predicting Wnt/β-catenin signal-activated HCC, which is considered to have an “immune cold microenvironment” and is primarily resistant to treatment with immune checkpoint inhibitors alone.

Abbreviations:
HCC, hepatocellular carcinoma; HCV, hepatitis C virus; JSH, Japan Society of Hepatology; US, ultrasonography; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; CT, computed tomography; MRI, magnetic resonance imaging; Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; mUICC, modified Union for International Cancer Control; BCLC, Barcelona Clinic Liver Cancer; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; TAE, transcatheter arterial embolization; HAIC, hepatic arterial infusion chemotherapy; MTAs, molecular-targeted agents; ICIs, immune checkpoint inhibitors; OS, overall survival; RFS, recurrence-free survival; B-TACE, Balloon-occluded TACE; mALBI, modified albumin-bilirubin; TACTICS, TACE therapy in combination with sorafenib; PFS, progression-free survival; APPLE, Asia-Pacific Primary Liver Cancer Expert; AEs, adverse effects; AI, artificial intelligence
STAGING

Clinical practice guidelines overview

The tumor staging systems for HCC in Japan include the modified Union for International Cancer Control (mUICC) staging system\(^\text{11}\) and the General Rules for the Clinical and Pathological Study of Primary Liver Cancer established by the Japan Liver Cancer Association (Fig. 1).\(^\text{12}\) The latter has been used for a nationwide follow-up survey of HCC in Japan, and the data is updated biannually.

Real-life practice

From a therapeutic point of view, the Barcelona Clinic Liver Cancer (BCLC) staging system is the most popularly used system in Japan and other countries. The system is useful for determining therapeutic options for HCC based on clinical information, including performance status, hepatic functional reserve, and tumor characteristics.

TREATMENT

Clinical practice guidelines overview

According to the Guidelines for Liver Cancer Treatment 2021, the treatment algorithm for HCC is based on five factors: hepatic functional reserve, extrahepatic metastasis, vascular invasion, tumor number, and tumor size (Fig. 2).\(^\text{8}\) For patients with Child-Pugh class A or B, the following three treatment options are recommended in the absence of extrahepatic metastases and vascular invasion: 1) If the number of tumors is 1–3 and the tumor diameter is <3 cm, hepatic resection or radiofrequency ablation (RFA) is recommended. If one tumor is present, hepatic resection is recommended as the first choice, regardless of the tumor diameter. 2) If the number of tumors is 1–3 and the tumor diameter is greater than 3 cm, hepatic resection is recommended as the first choice, followed by transcatheter arterial chemoembolization/embolization (TACE/TAE) as the second choice. 3) If the number of tumors is four or more, TACE is recommended as the first choice, followed by hepatic arterial infusion chemotherapy (HAIC) or systemic drug therapies, using molecular-

---

**Figure 1.** The staging system for HCC according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (the Japan Liver Cancer Association). HCC, hepatocellular carcinoma; Meta, metastasis.
targeted agents (MTAs) and ICIs as the second choice. Systemic drug therapy is recommended for patients with HCC, Child-Pugh class A cirrhosis, and extrahepatic metastases.\(^8\) Liver transplantation (LT) is recommended for HCC patients with Child-Pugh class C cirrhosis if they are within the Milan criteria (three or fewer tumors and ≤3 cm in diameter, or one tumor ≤5 cm in diameter) or 5-5-500 criteria (five or fewer tumors, ≤5 cm in diameter, and AFP ≤500 ng/mL) and the patient is under 65 years of age. These criteria for liver transplantation are different from those used in the BCLC staging system. Palliative care is recommended for HCC patients with Child-Pugh class C cirrhosis who are unsuitable for LT.\(^8\)

**Real-life practice**

**Curative treatments**

Hepatic resection, LT, and locoregional therapy (ethanol injection, microwave coagulo-necrotic therapy, and RFA) for HCC are considered to be curative treatments in Japan.\(^{13,14}\) According to Kudo et al.\(^{15}\), 40.3% of patients were treated with hepatic resection or LT and 21.1% of patients were initially treated with locoregional therapy. The median overall survival (OS) of patients who underwent hepatic resection was 92.5 months, and the 5- and 10-year survival rates were 66.7% and 40.8%, respectively. Conversely, the median OS of patients who underwent RFA was 75.8 months, and the 5- and 10-year survival rates were 61.7% and 28.0%, respectively.\(^{15}\) According to the results of the SURF trial,\(^{16}\) a domestic randomized controlled trial that compared the OS following hepatic resection and that following RFA, RFA is now considered equally effective to hepatic resection. In this trial, recurrence-free survival (RFS) did not differ significantly between groups. The median RFS was 3.5 years in the hepatic resection group and 3.0 years in the RFA group (hazard ratio [HR], 0.92; 95% confidence interval [CI], 0.67–1.25; \(P=0.58\)). Thus, RFS did not differ significantly following hepatic resection or RFA in patients with the largest HCC diameter ≤3 cm and ≤3 HCC nodules.

In addition to intrahepatic HCC lesions, RFA also appears to be effective in patients with pulmonary HCC metastases.\(^{17}\)

---

**Figure 2.** The treatment algorithm for HCC according to the Guidelines for Liver Cancer Treatment 2021 in Japan. The algorithm is based on five factors: hepatic functional reserve, extrahepatic metastasis, vascular invasion, tumor number, and tumor size. *1. Assessment based on liver damage is recommended in the case of hepatectomy; *2. Patients with Child–Pugh class A only; *3. Patients aged ≤65 years; and *4. No extrahepatic metastasis or vascular invasion. Five or fewer tumors, size ≤5 cm in diameter, and AFP ≤500 ng/mL. HCC, hepatocellular carcinoma; HAIC, hepatic arterial infusion chemotherapy; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; AFP, alpha-feto-protein.
TACE

TACE is the standard of care for patients with intermediate-stage HCC and has been used worldwide. Balloon-occluded TACE (B-TACE), a type of TACE, was established in Japan by Irie et al. Recently, it has been reported that substantially longer local recurrence-free periods were observed after B-TACE than after conventional TACE and other types of TACE. However, repeated TACE is associated with a high rate of treatment failure, worsening liver function, and poor prognosis. Therefore, the concept of unsuitable TACE for intermediate-stage HCC was recently proposed. Unsuitable TACE is generally defined as follows: 1) likely to develop TACE failure/refractoriness, 2) likely to develop Child-Pugh class B liver function after TACE, and 3) unlikely to respond to TACE. Unsuitable TACE includes patients, who exceed the up-to-seven criteria, those, who have liver function classified as modified albumin-bilirubin (mALBI) grade 2b, or those, who have HCC other than the simple nodular type.

Recently, several studies have reported that combining MTA and TACE therapy significantly improved OS compared to that associated with TACE alone in patients with unresectable HCC. The TACE therapy in combination with sorafenib (TACTICS) trial showed that sorafenib-TACE sequential therapy yielded significantly longer progression-free survival (PFS) compared to that provided by TACE alone (25.2 months vs. 13.5 months, HR, 0.59; 95% CI, 0.41–0.87; P<0.0001). Moreover, Kudo et al. reported the beneficial effects of lenvatinib on the OS rate in patients with intermediate-stage HCC showing large or multinodular tumors exceeding the up-to-seven criteria. To improve survival in patients with intermediate-stage HCC unsuitable for TACE, this strategy was approved at a consensus meeting of the Asia-Pacific Primary Liver Cancer Expert (APPLE) Association and the JSH. Furthermore, upfront systemic therapy was also recently recommended for patients who are TACE-unsuitable in the European Society for Medical Oncology clinical practice guidelines and the American Association for the Study of Liver Diseases.

HAIC

HAIC was the standard therapy for advanced HCC in Japan. However, the role of HAIC has been reconsidered due to recent progress in systemic therapies. Combination therapies of HAIC with systemic therapies have recently attracted attention. Ikeda et al. reported the effectiveness of combining cisplatin HAIC monotherapy with sorafenib in a randomized phase 2 clinical trial. Kudo et al. reported a randomized phase 3 clinical trial comparing sorafenib monotherapy with sorafenib plus a low-dose cisplatin/5-fluorouracil (FP) HAIC regimen. In that study, low-dose FP plus sorafenib did not show any additive survival benefits in all enrolled patients, compared to that with sorafenib alone. However, the combination therapy was significantly more effective in patients with advanced HCC with severe portal vein tumor thrombus. Combination therapy using HAIC and lenvatinib has also been reported. Shimose et al. reported the effectiveness of combination therapy using a New FP HAIC regimen (Lipiodol-suspended FP) and lenvatinib. Their study revealed that alternating therapy with the New FP regimen and lenvatinib significantly prolonged the administration period of lenvatinib and patient survival. Sequential therapy from HAIC to systemic therapy or systemic therapy to HAIC is also challenging. Kondo et al. reported a clinical trial of sequential HAIC and sorafenib treatment. However, the trial showed that this treatment did not improve the survival benefits compared to that with sorafenib alone. Therefore, the establishment of multidisciplinary therapeutic strategies for the management of advanced HCC remains an unmet medical need in Japan.

Radiation and particle therapies

Since April 2022, particle therapies using protons and carbon ions have been applied to large (≥4 cm) and difficult-to-resect HCCs and are covered by health insurance in Japan. Further studies are needed to establish solid evidence for both stereotactic body radiation therapy and particle therapy.

Systemic therapies

Compared to those in lenvatinib therapy, atezolizumab plus bevacizumab therapy prolonged PFS preserved hepatic functional reserve and resulted in lower rates of severe adverse effects (AEs). Thus, combination therapy is often selected as the first-line treatment, except for patients who should avoid immunotherapies or those with impaired liver function. Kudo recently advocated the concept of ‘ABC conversion’ with the aim of a cancer-free/treatment-free status. This concept proposes using atezolizumab plus bevacizumab followed by curative conversion for patients with advanced HCC. The conversion rate in an atezolizumab plus bevaciz-
zumab treatment group was reported to be higher than that in a lenvatinib treatment group (8.6% vs. 1.9%, \( P=0.007 \)), and resulted in a high conversion rate (35% [38/110]) with 22% of cases achieving a cancer-free/treatment-free status after receiving atezolizumab plus bevacizumab treatment. To determine the true therapeutic effects of atezolizumab plus bevacizumab treatment, it is important to understand the discrepancy between radiological findings and biochemical responses.

Lenvatinib is also used as a front-line treatment because of the accumulated clinical evidence and innovations, such as the weekends-off method, which is an attempt to reduce AEs while maintaining therapeutic efficacy. Consequently, 66.7% of patients who were intolerant to prior lenvatinib therapy completed the weekends-off strategy with an improved therapeutic response in 61.5% of those patients. For patients with lenvatinib-refractory HCC, sorafenib is a possible treatment option. Fortunately, lenvatinib-sorafenib sequential therapy is available in Japan under the insurance system. However, it is difficult to switch to sorafenib in patients who have discontinued lenvatinib due to AEs such as palmar-planter erythrodysesthesia. Further studies are needed to optimize sequential systemic drug therapy for lenvatinib-refractory HCC. Furthermore, combining lenvatinib with TACE to enhance antitumor effects has a significant survival benefit, particularly in patients with non-viral HCC. Additionally, in the final analysis for the phase II trial (TACTICS-L trial), the combination of TACE and lenvatinib showed promising therapeutic efficacy in patients with unresectable HCC.

Although there is no significant evidence for second-line treatment in patients in whom atezolizumab plus bevacizumab has failed, ramucirumab and lenvatinib have shown promising results as second-line treatments. Cabozantinib may have beneficial effects in patients who have received one or two prior systemic anticancer therapies for advanced HCC with subsequent radiographic progression. The determination of ideal sequential systemic chemotherapy, including atezolizumab plus bevacizumab, as front- or later-line treatment is complex and controversial; therefore, further evidence should be accumulated.

**DISCUSSION**

From a global perspective, there are two major trends in the clinical management of HCC. One is the futuristic challenges in diagnostics, including artificial intelligence (AI), and the other is the further advancement of combined immunotherapy for advanced HCC. The introduction of AI for diagnosing HCC will not only improve diagnostic accuracy but also lead to accurate prediction of treatment efficacy in collaboration with multi-omics analysis. Thus, AI will integrate various types of images and biological information and provide real-time information to determine whether the tumor immune microenvironment is hot or cold, determine the degree of responsiveness to MTAs, and predict endogenous genetic changes in the tumors in the near future. In Japan, results from exploratory research on AI diagnosis of HCC are emerging.

The emergence of combined immunotherapy for advanced HCC has brought about a paradigm change. However, in the absence of appropriate biomarkers to predict therapeutic efficacy, achieving a cure with systemic therapy alone is challenging. Although chimeric antigen T-cell therapies and other therapies are being developed, the most urgent need is identifying the optimal solution for second-line and later therapies using currently available drugs. In this regard, a prospective observational registry study called the PRISM study is underway in Japan to determine the optimal systemic therapy to follow atezolizumab plus bevacizumab therapy for unresectable HCC patients. The results of the PRISM study are expected to help manage the chaotic situation in determining the optimal second-line systemic treatment, according to the current guidelines in Japan.

**Authors’ contribution**

TK drew up the basic plan for writing this paper and HI proposed the role assignment. MN wrote the Curative treatment part, SS wrote the TACE part, HI wrote the HAIC part, HS wrote the Systemic therapies description, and HK wrote the ABSTRACT, INTRODUCTION, SURVEILLANCE AND DIAGNOSIS, STAGING, DISCUSSION, and TREATMENT guideline overview. HK integrated all described parts and refined the article. All authors participated in a critical discussion.
Conflicts of Interest

H.K. received lecture fees from Chugai Pharmaceutical Co. Ltd. and Eisai Co. Ltd. T.K. 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., and AbbVie GK. T.K. received research funding from Eisai Co. Ltd. The other authors have no conflicts of interest pertaining to this study.

REFERENCES


glypican-3 (GPC-3)-specific chimeric antigen receptor (CAR) and soluble IL-15 display robust antitumor efficacy against hepatocellular carcinoma. J Immunother Cancer 2021;9:e003441.
Overview of Asian clinical practice guidelines for the management of hepatocellular carcinoma: An Asian perspective comparison

Yuri Cho*, Bo Hyun Kim*, and Joong-Won Park

Center for Liver and Pancreatobiliary Cancer, National Cancer Center, Goyang, Korea

Hepatocellular carcinoma (HCC) is highly prevalent and the third most common cause of cancer-related death in Asia. In contrast to the West, the main etiology of HCC in many Asian countries except Japan is chronic hepatitis B virus infection. Differences in the major causes of HCC lead to significant clinical and treatment differences. This review summarizes and compares guidelines on managing HCC from China, Hong Kong, Taiwan, Japan, and South Korea. From oncology and socio-economic perspectives, factors such as underlying diseases, staging methods, government policies, insurance coverage, and medical resources contribute to varying treatment strategies among countries. Furthermore, the differences in each guideline are fundamentally caused by the lack of incontrovertible medical evidence, and even existing results of clinical trials can be interpreted differently. This review will provide a complete overview of the current Asian guidelines for HCC in recommendations and in practice. (Clin Mol Hepatol 2023;29:252-262)

Keywords: Hepatocellular carcinoma; Asia; Practice guideline; Chronic hepatitis B

INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly prevalent cancer in Asia when compared to the West and is the third most common cause of cancer-related death in the Asia-Pacific region.1,2 Despite a hepatitis B virus (HBV) vaccination program, the most prevalent etiology of HCC in Asia except for Japan is still chronic hepatitis B infection, followed by hepatitis C virus (HCV) infection.3

In contrast to other malignancies, HCC has a high-risk factor for occurrence, making surveillance testing important. Due to the difficulty and risk of tissue diagnosis, it is often confirmed through imaging tests alone. Most patients have chronic liver disease, so treatment strategies are determined based on their underlying conditions, such as liver disease. In addition, liver transplantation and transarterial chemoembolization (TACE) show big differences in treatment compared to other cancers. Because the underlying liver disease remains even after radical surgery, the recurrence rate of HCC is higher than in other cancers, which leads to consideration for subsequent treatment and a multidisciplinary approach.

After the Barcelona Clinic Liver Cancer group developed...
combined guidelines for a staging system and treatment strategies for HCC for the first time,^4,5 evidence-based or consensus-based guidelines began to be developed in South Korea,^6 Japan,^7,8 China,^9 and other countries. Each country had developed similar but different guidelines. Several factors contributed to these differences, such as the primary cause of HCC, prevalence, and characteristics of underlying diseases, varying staging systems, government and medical insurance reimbursement policies, medical resources, compliance of doctors and patients, and cultural differences. The guidelines developed in different countries differ fundamentally because there is a lack of concrete medical evidence, and even existing results of clinical trials can be interpreted differently. Fortunately, after 20 years of development and revision, various guidelines tend to converge similarly while influencing each other.

In this review, we summarized and compared the current guidelines of HCC in Asian countries including surveillance strategies, diagnostic modalities, staging systems, and treatment modalities, as well as locoregional and surgical treatment modalities. Additionally, we discussed the future management of HCC in Asia

**SURVEILLANCE**

**Clinical practice guideline overview**

Most Asia HCC practice guidelines recommend regular surveillance at six-month intervals for HCC in high-risk groups, including patients with chronic hepatitis B or C, and liver cirrhosis at a 6-month interval. In China,^10 the aMAP (age-Male-ALBI-Platelets) score was newly added in the 2022 Chinese guideline to help discriminate high-risk groups. Taiwanese guidelines recommend the HCC surveillance interval with a range of 6–12 months. In Japan, liver ultrasonography (US) plus alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP) and AFP-L3 testing are recommended every six months for the high-risk group (HBV/HCV infection, cirrhosis of other etiologies) and every three to four months for the extremely high-risk group (HBV/HCV-related cirrhosis).^11

All guidelines recommend the combined use of serum AFP and liver US except the Asian Pacific Association for the Study of the Liver (APASL) guideline.^12 APASL guidelines suggest that AFP is not recommended as a confirmatory test in small HCCs, and the cut-off value of AFP should be set at 200 ng/mL for the surveillance program. APASL and Taiwanese guidelines recommend imaging modalities including contrast-enhanced ultrasonography (CEUS). Korean,^13 Hong Kong,^14 and Taiwanese^15 guidelines recommend dynamic contrast-enhanced computed tomography (CT) or dynamic contrast-enhanced magnetic resonance imaging (MRI) as an alternative to suboptimal US assessment.

**Real-life practice**

Many efforts and strategies are improving the adherence rate to HCC surveillance recommendations in Asia. The Korean government has initiated the National Liver Cancer Screening Program since 2003. The Bureau of National Health Insurance in Taiwan^17 started a medical care improvement plan for patients with chronic HBV and HCV infection since 2000. In China, lack of government-supported programs to cover surveillance for more population also contributed to a low diagnostic rate of early HCC.^18 Timely diagnosing of early-stage HCC and curative treatment are still suboptimal in Asia. According to the Korean National Health Insurance Service database, only 52.7% of high-risk individuals participated in the national liver cancer surveillance program during 2003–2015. More efforts to increase the adherence rate to surveillance should be performed in Asia. Also, alternative screening tools, including abbreviated MRI^19 should be validated as screening methods for HCC to increase sensitivity.

Abbreviations:

AFP, alpha-fetoprotein; ALPPS, Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy; aMAP, age-Male-ALBI-Platelets score; APASL, Asian Pacific Association for the Study of the Liver; CEUS, contrast-enhanced ultrasonography; CHB, chronic hepatitis B; CT, computed tomography; DCP, des-gamma-carboxy prothrombin; DEB, drug-eluting beads; Gd, Gadolinium; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IO, immune-oncology; MRI, magnetic resonance imaging; SBRT, stereotactic body radiation therapy; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; TKI, tyrosine kinase inhibitor; UCSF, University of California San Francisco; US, ultrasonography
DIAGNOSIS

Clinical practice guideline overview

Korean, Japanese, and Taiwanese guidelines recommend the diagnosis of HCC according to pathology or using typical radiologic hallmarks of HCC through dynamic contrast-enhanced CT or MRI, for a ≥1 cm nodule detected by surveillance among high-risk patients. In China, the diagnosis of HCC can be made if nodules >2 cm among high-risk patients with typical features on any of the three imaging modalities including multiphasic dynamic CT, MRI or Gadolinium (Gd)-EOB-DTPA-enhanced MRI, and CEUS. The diagnosis of nodules ≤2 cm in China can be established with typical imaging features on at least two modalities. APASL guideline also suggest that CEUS is useful and is as sensitive as dynamic CT or dynamic MRI in diagnosis of HCC. Korea and APASL guidelines highlight the importance of a combined interpretation of the dynamic and hepatobiliary phases of the Gd-EOB-DTPA-enhanced MRI with diffusion-weighted imaging.

Real-life practice

In Korea, radiological hallmarks include arterial phase hyperenhancement with washout appearance in multiphasic CT or MRI with extracellular contrast agent or MRI with liver-specific contrast and CEUS. A diagnosis of “probable” HCC can be made by applying ancillary imaging features. APASL guidelines are frequently referenced by clinicians in Hong Kong. In Taiwan, physicians have adopted a more aggressive attitude toward active tumor biopsy, due to the booming numbers of immunotherapy combination trials in HCC with 48.2% of HCC diagnoses supported by pathology or cytology in 2019. In China, diagnostic accuracy is given priority over sensitivity due to the unbalanced distribution of medical resources. Therefore, confirmation of HCC diagnosis with two imaging modalities for nodules of 1–2 cm was adopted in China.

STAGING

Clinical practice guideline overview

Korea and Japan guidelines adopted the modified Union for International Cancer Control (mUICC) stages as the primary staging system (Fig. 1). In Taiwan, the Barcelona Clinic of Liver Cancer (BCLC) staging system is the most commonly used staging system in terms of prognostic prediction. Hong Kong consensus guidelines suggest the Hong Kong Liver Cancer (HKLC) staging system (Fig. 2) as the staging system of choice, which was developed in 2014 with data from 3,856 HCC patients in Hong Kong. HKLC staging includes the Eastern Cooperative Oncology Group status, Child-Pugh score, the presence of extravascular invasion or metastasis, tumor size, and number of tumor nodules. In China, the China liver cancer staging system incorporating tumor characteristics, liver function, and performance status, which is similar to the BCLC system, was established in 2017. APASL guideline also suggest a treatment algorithm (Fig. 4) according to extrahepatic metastasis, Child-Pugh class, resectability, macrovascular invasion, tumor number, or size.

<table>
<thead>
<tr>
<th>mUICC stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IV A</td>
<td>T4</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T1, T2, T3, T4</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>IV B</td>
<td>T1, T2, T3, T4</td>
<td>N0, N1</td>
<td>M1</td>
</tr>
</tbody>
</table>

Criteria
1. Number of tumor: solitary
2. Diameter of the largest tumor ≤2 cm
3. No vascular or bile duct invasion
4. T1: 3 criteria fulfilled
5. T2: 2 criteria fulfilled
6. T3: 1 criterion fulfilled
7. T4: none

Figure 1. Modified Union for International Cancer Control (mUICC) stages.
Figure 2. Staging and preferred treatment in the Hong Kong Liver Cancer Staging (HKLC) system. EVM, extravascular metastasis; ECOG, Eastern Cooperative Oncology Group; TACE, transarterial chemoembolization.

Figure 3. China liver cancer staging (CNLC) system. HCC, hepatocellular carcinoma; PS, performance status.
Real-life practice

Korea guidelines\(^{14}\) adopt mUICC staging as the primary system, however, the BCLC staging system and American Joint Committee on Cancer (AJCC)/UICC TNM staging system also serve as complementary systems. Although the BCLC staging system is the most used staging system in Taiwan, the treatment algorithm suggested by BCLC staging system does not reflect the true daily practice in Taiwan and most Asian countries where diverse locoregional therapy and systemic therapies are available.

TREATMENT

Resection

Clinical practice guideline overview

Most Asian guidelines recommend surgical resection for single HCC of any size, as in the European or American guidelines.\(^{10,12,14,15,17,25,26}\) Most Asian guidelines also recommend surgical resection for multiple tumors if lesions are localized.\(^{10,14,15,17,27,28}\) Chinese guidelines suggest that surgical resection can be considered if tumors with more than four lesions or vascular invasion are confined in the same segment or lobe.\(^{10,28}\) Hong Kong guidelines recommend surgical resection for solitary or multifocal lesions confined to the liver.\(^{15,27}\) Taiwanese guidelines suggest surgical resection for multiple tumors in one lobe of the liver.\(^{17}\) Even if tumors invade portal vein, surgical resection may be considered in selected patients according to Asian guidelines.\(^{10,12,15,17}\) In other Asian countries except Korea, it is thought that the guidelines were developed mainly by surgeons based on consensus, and accordingly, the indications for surgery seem to have become wider.

For patients with inadequate future liver remnants, Chinese and Hong Kong guidelines suggest portal vein embolization or Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS), whereas American guidelines suggest portal vein embolization or transarterial radioembolization (TARE) to increase the chance of resection.\(^{10,15,26}\)

Real-life practice

Most Asian guidelines advocate surgical resection in more
expanded indications.\textsuperscript{10,14,15,17,27-30} Even in HCC patients with portal vein invasion, surgical resection has been performed in China, Hong Kong, and Taiwan.\textsuperscript{27,28,30} Since surgical resection is more aggressively performed, several methods to improve resectability, such as portal vein embolization or ALPPS, were mentioned. However, ALPPS is limited and is only performed in specialized centers in Hong Kong.\textsuperscript{27} Other than these methods, multi-modal treatment strategies such as systemic therapy, hepatic arterial infusion chemotherapy, or TACE have been performed to improve resectability.\textsuperscript{28}

Liver transplantation

**Clinical practice guideline overview**

Most Asian guidelines recommend liver transplantation as the primary treatment in patients with poor liver function.\textsuperscript{10,14,15,17,25,26,29} Patient selection criteria differ from country to country. Taiwanese, Japan and Korean guidelines adopt the Milan criteria as the major criteria, while Chinese and Hong Kong guidelines adopt the University of California San Francisco (UCSF) criteria.\textsuperscript{10,12,14,15,17,25,26,29} Asian countries are confronting a deceased donor shortage, and it is a critical barrier to liver transplantation. Most Asian guidelines mentioned living donor liver transplantation.\textsuperscript{10,12,14,15}

**Real-life practice**

Liver transplantation is an ideal treatment for patients with HCC and liver cirrhosis. However, organ shortage often precludes liver transplantation, and most Asian guidelines stated living donor liver transplantation.\textsuperscript{10,12,14,15} Organ shortage in Taiwan leads to a salvage transplantation strategy; surgical resection is performed as the primary treatment, followed by transplantation in the case of recurrence of liver failure.\textsuperscript{48} In Hong Kong, Japan and Korea, living donor liver transplantation is the predominant type of transplant surgery.\textsuperscript{12,14,27} Sometimes, more expanded criteria beyond the Milan or UCSF criteria, or biomarker-based criteria can be applied for living donor liver transplantation.\textsuperscript{12,14,28}

Ablation

**Clinical practice guideline overview**

Most Asian guidelines recommend local ablation for very early- or early-stage HCCs ≤3 cm.\textsuperscript{10,12,14,15,17} Chinese guidelines also recommended local ablation for solitary HCC ≤5 cm as a curative treatment.\textsuperscript{10} Japanese guidelines recommend radiofrequency ablation for HCC with ≤3 cm and ≤3 nodules, and surgical resection for solitary HCC >3 cm as a primary choice.\textsuperscript{12,31} Hong Kong and Taiwanese guidelines recommend local ablation in unsuitable cases for resection.\textsuperscript{15,17} Microwave ablation as well as radiofrequency ablation are recommended as local ablation modalities.

**Real-life practice**

Local ablation therapies show similar overall survival and slightly higher local tumor progression rates compared with surgical resection. Larger tumor size or high-risk locations may lead to higher local tumor progression rates. Artificial ascites or pleural effusion infusion may be considered to overcome these limitations.\textsuperscript{10,12,17,30} In China, local ablation in combination with TACE is considered for larger tumors.\textsuperscript{10,28}

Transarterial therapy

**Clinical practice guideline overview**

TACE is a widely used therapeutic modality for intermediate-stage HCC worldwide. However, each country’s staging method is slightly different; therefore, each country’s indications seem different from the intermediate stage of BCLC. Transarterial therapy using new materials such as drug-eluting beads (DEB), or radioisotope (yttrium-90) has been introduced. DEB-TACE and TARE offer a low incidence of postembolization syndrome, one of the painful adverse events of conventional TACE. Most Asian guidelines recommend TACE as a major treatment modality for intermediate-stage HCC without major vascular invasion or extrahepatic spread.\textsuperscript{10,12,14,15,17} Hong Kong guidelines suggest TACE in HCC with portal vein invasion either alone (segmental portal vein invasion) or in combination with radiotherapy.\textsuperscript{15,27} Taiwanese guidelines suggest that TACE can be considered in HCC with portal vein invasion in combination with targeted therapy, radiotherapy, TARE, or hepatic arterial infusion chemotherapy.\textsuperscript{17,30} Japanese guidelines recommend TACE for HCCs with 2–3 tumors of ≥3 cm in diameter or ≥4 tumors primarily.\textsuperscript{12,31} They also suggest conventional TACE for small tumors and DEB-TACE for large tumors since conventional TACE is theoretically more effective than DEB-TACE for small HCCs and DEB-TACE shows milder adverse events.\textsuperscript{12,31} Korean guidelines
also recommend drug-eluting bead TACE as an alternative treatment to conventional TACE in HCCs ≥3 cm since the local tumor response of DEB-TACE was significantly lower than that of conventional TACE in HCCs <3 cm, \(^{14,32}\) and TARE because of offering a better quality of life and lower incidence of postembolization syndrome.\(^{14}\)

**Real-life practice**

In real clinical practice for many Asian countries, TACE has been widely adopted as a primary option for early, intermediate, and advanced-stage HCC. Implementation of newer methods such as DEB-TACE and TARE depends on the policy of each government and the economic burden. In Taiwan, DEB-TACE and TARE are not reimbursed by the NHI program yet.\(^{10}\) TARE is also less frequently performed in China and Hong Kong.\(^{27,28}\) The costs of both DEB-TACE and TARE are still high since they are reimbursed partly by the government in Korea\(^{14}\); however, the use of TARE is increasing.

**Systemic therapy**

**Clinical practice guideline overview**

Most guidelines commonly recommend atezolizumab plus bevacizumab, sorafenib, and lenvatinib as a first-line option (Table 1).\(^{10,11,14,15,29,31,33}\) Hong Kong guidelines also recommended nivolumab for patients who have a contraindication to antiangiogenic therapies or tyrosine kinase inhibitors (TKIs) as first-line treatment.\(^{15}\) Korean guidelines included tremelimumab plus durvalumab since the guidelines were most recently released (2022).\(^{14}\) Chinese guidelines adopted many systemic agents developed in China: donafenib and sintilimab plus bevacizumab biosimilar (Byvasda), apatinib, camrelizumab, and tislelizumab.\(^{29}\) As first-line treatment, Chinese guidelines recommended atezolizumab plus bevacizumab, lenvatinib, sorafenib, donafenib, sintilimab plus bevacizumab biosimilar (Byvasda), and FOLFOX.\(^{10,29}\)

As a second-line option, regorafenib, cabozantinib, ramucirumab, nivolumab with or without ipilimumab, and pembrolizumab were recommended in Hong Kong, Taiwan, and Korea.\(^{27,14,15}\) Chinese guidelines recommended regorafenib, apatinib, camrelizumab, and tislelizumab as second-line treatment.\(^{10,29}\)

Japanese guidelines recommend regorafenib, cabozantinib, and ramucirumab as a second-line option. Japanese guidelines also recommend sorafenib or lenvatinib for pa-
tients who did not experience sorafenib or lenvatinib as a first-line option, respectively.\textsuperscript{31}

Real-life practice
A couple of new systemic agents have been introduced recently. Major study results as well as the approval and/or reimbursement status incorporate into the real clinical practice.

First-line immunotherapy is not reimbursed yet in Taiwan; therefore, atezolizumab plus bevacizumab or lenvatinib plus pembrolizumab were given based on shared decision-making.\textsuperscript{29} In Hong Kong, nivolumab plus ipilimumab is also given as a first-line therapy.\textsuperscript{27} In China, PD-1/PD-L1 inhibitors plus TKIs have been in wide use in real clinical practice.\textsuperscript{28} In Korea\textsuperscript{14}, only one option among atezolizumab plus bevacizumab, lenvatinib, and sorafenib is reimbursed as a first-line option; atezolizumab plus bevacizumab is most commonly chosen nowadays.

Most second-line treatments are recommended for sorafenib failure. Recommendations on subsequent therapy after atezolizumab plus bevacizumab or lenvatinib is based on experts’ opinion.\textsuperscript{14,27,28,30} In Hong Kong guidelines, regorafenib, cabozantinib, ramucirumab or unused TKI, and immunotherapy could be considered after lenvatinib failure.\textsuperscript{15,27} Nivolumab with or without ipilimumab and pembrolizumab can be considered after approved TKIs (lenvatinib, sorafenib) in Taiwan.\textsuperscript{30} In Japan, sorafenib or lenvatinib is recommended after atezolizumab plus bevacizumab failure.\textsuperscript{12,31} Korean guidelines\textsuperscript{14} mentioned that unused TKI or IO drugs that do not share mechanisms of action can be given to lenvatinib or first-line IO-based doublet therapy failures; however, all subsequent agents are not reimbursed as second-line treatment after lenvatinib or atezolizumab plus bevacizumab as of March 2022.

External beam radiation therapy

Clinical practice guideline overview
Most Asian guidelines recommend external beam radiation therapy in selected patients, while Western guidelines suggest that external beam radiotherapy is under investigation since there is a lack of robust evidence. Radiotherapy can be given as an alternative to surgical resection or locoregional therapeutic modalities (local ablation or TACE) or as a palliative modality. In China, stereotactic body radiation therapy (SBRT) is indicated for patients with solitary lesions or multiple tumors ≤3 cm who are ineligible for curative treatment.\textsuperscript{10} Radiotherapy can be given to patients with multiple HCCs in combination with TACE or tumor thrombus or oligometastasis.\textsuperscript{10,28} Hong Kong guidelines suggest SBRT as an alternative to other liver-directed therapies and SBRT can also be given in combination with TACE for large tumors (>5 cm).\textsuperscript{15} Taiwanese guidelines suggest radiotherapy for HCCs ineligible or inaccessible to local ablation or TACE, or HCCs with portal vein invasion.\textsuperscript{17,30} Radiotherapy may also be considered for symptomatic metastasis or oligometastasis.\textsuperscript{15} Korean guidelines recommend radiotherapy patients with HCC unsuitable for hepatic resection, transplantation, or locoregional therapy.\textsuperscript{14} Radiotherapy can be additionally performed for HCC showing incomplete response to TACE or with portal vein invasion.\textsuperscript{14} Korean guidelines also recommend proton beam radiotherapy since it showed comparable local tumor control and overall survival compared with radiofrequency ablation in HCCs ≤3 cm.\textsuperscript{14,34}

Real-life practice
Radiotherapy has been widely used in Asia as an alternative therapy to curative treatment or in combination with other locoregional therapies. Radiotherapy is versatile and the favored treatment modality.\textsuperscript{27,28,30} SBRT can deliver ablative radiation dose in low fractions and SBRT for unresectable HCC either alone or in combination with TACE is increasing in Hong Kong.\textsuperscript{7} Also in Korea, radiotherapy is used for HCCs unsuitable for surgical resection, local ablation, or TACE.\textsuperscript{14,31} Radiotherapy may also be performed in combination with TACE or systemic therapy.\textsuperscript{14,35}

DISCUSSION

HCC is a heterogeneous and complex disease in which multidisciplinary approaches are necessary to optimize management. In contrast to other cancers, the regional differences in etiology-dependent tumor biology and socio-medical resources make it impractical to have a globally universal guideline for all patients with HCC. Asian HCC practice guidelines are similar but different from each other because of similar main causes (i.e., HBV infection) but different interpretations of results from clinical trials secondary to a lack of concrete, high-level evidence. Therefore, it is serious to acknowledge these challenges and devise effective strategies
to overcome them, enhance the design of upcoming clinical trials, and finally, improve the outcomes of HCC patients worldwide. Since the liver is an immune-tolerant organ,\textsuperscript{36,37} it was assumed that immune checkpoint inhibitor therapy of HCC would be disadvantageous; however, recently, atezolizumab plus bevacizumab\textsuperscript{38} and durvalumab plus tremelimumab\textsuperscript{39} have succeeded in clinical trials, making them available for advanced patients in some countries, and recommended in the most consistently universal guidelines. Like such achievements, multidisciplinary efforts to optimize treatment and improve clinical trial designs are ultimately necessary.

Despite some mutual criticism and rejection, Eastern and Western physicians have learned from each other regarding improving patient outcomes. A better understanding of differences in oncological background and socio-economic resources in different countries can help make better multinational clinical trials and provide better outcomes, from which more concrete evidence and an indisputable recommendation could be developed in most countries. Because consensus-based recommendations are more influenced by socio-economic policies, medical resources, leading physicians, compliance of patients, and culture, it is critical to remember those influences when understanding the recommendations.

Improving the survival rates of patients with HCC across all stages is a crucial goal that can be achieved by developing more effective biomarkers for prediction and personalized therapeutic approaches based on well-designed multinational clinical trials.

**Authors’ contribution**

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

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

2. Kim BH, Park JW. Epidemiology of liver cancer in South Korea.
Hepatol 2022;28:583-705.
17. Surveillance group; Diagnosis group; Staging group; Surgery group; Local ablation group; TACE/TARE/HAI group, et al. Management consensus guideline for hepatocellular carcinoma: 2016 updated by the Taiwan Liver Cancer Association and the Gastroenterological Society of Taiwan. J Formos Med Assoc 2018;117:381-403


Yuri Cho, et al.
Asian HCC practice guidelines

261

The role of different viral biomarkers on the management of chronic hepatitis B

Lung-Yi Mak1,2, Rex Wan-Hin Hui1, James Fung1,2, Wai Kay Seto1,2, and Man-Fung Yuen1,2

1Department of Medicine, School of Clinical Medicine and 2State Key Laboratory of Liver Research, The University of Hong Kong, Hong Kong

Chronic hepatitis B infection is a major public health challenge. With the advancement in technology, various components of the viral cycle can now be measured in the blood to assess viral activity. In this review article, we summarize the relevant data of how antiviral therapies impact viral biomarkers, and discuss their potential implications. Viral nucleic acids including hepatitis B virus (HBV) double-stranded deoxy-ribonucleic acid (DNA) and to a lesser extent, pre-genomic RNA, are readily suppressed by nucleos(t)ide analogues (NUCs). The primary role of these markers include risk prediction for hepatocellular carcinoma (HCC) and risk stratification for partial cure, defined as off-therapy virological control, or functional cure, defined as hepatitis B surface antigen (HBsAg) seroclearance plus undetectable serum HBV DNA for ≥6 months. Viral translational products including hepatitis e antigen, quantitative HBsAg and hepatitis B core-related antigen can be reduced by NUCs and pegylated interferon α. They are important in defining disease phase, delineating treatment endpoints, and predicting clinical outcomes including HCC risk and partial/functional cure. As the primary outcome of phase III trials in chronic hepatitis B is set as HBsAg seroclearance, appropriate viral biomarkers can potentially inform the efficacy of novel compounds. Early viral biomarker response can help with prioritization of subjects into clinical trials. However, standardization and validation studies would be crucial before viral biomarkers can be broadly implemented in clinical use. (Clin Mol Hepatol 2023;29:263-276)

Keywords: Chronic hepatitis B; Hepatitis B core antigen; Viremia; Treatment outcome
modulation. These mechanisms are being further explored to look for novel drug candidates to treat CHB infection. In addition, newer viral biomarkers have been identified to help evaluate treatment response and determine prognosis among treated patients. In this review, we will discuss the profile and potential applications of various blood-based viral biomarkers in CHB patients receiving antiviral therapy.

THE HBV VIRAL CYCLE

To date, there are no effective treatments to clear HBV from the infected liver due to the peculiar mechanisms of the viral cycle (Fig. 1). HBV is an enveloped, hepatotropic partially double-stranded deoxy-ribonucleic acid (DNA) virus. A mature HBV virion is fully encapsidated and contains relaxed circular (rc) DNA of approximately 3.2 kilobase pairs. Following entry into the hepatocytes via interaction with the sodium taurocholate co-transporting polypeptide, the rcDNA is imported to the nucleus and is repaired by host cell DNA repair machinery and converted to covalently closed circular DNA (cccDNA), which serves as the template for viral transcription. The HBV genome consists of four overlapping open reading frames, which give rise to viral transcripts that include the pre-genomic RNA (pgRNA) and messenger RNAs for subsequent translation of viral proteins: hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antigen (HBcAg), X protein (HBx), and HBV polymerase. The pgRNA is packaged in a capsid made from HBcAg, a process also known as encapsidation, followed by reverse transcription into rcDNA and to a lesser extent, double-stranded linear DNA (dslDNA). These viral genomes are then enveloped.

**Figure 1.** Viral cycle of hepatitis B virus. Those highlighted in asterisks are detectable in the bloodstream and can be used as viral biomarkers. These include HBsAg, HBeAg, HBcAg, HBV DNA and pgRNA. cccDNA, covalently closed circular DNA; dslDNA, double-stranded linear DNA; HBV, hepatitis B virus; HBcAg, hepatitis core antigen; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; mRNA, messenger RNA; NTCP, sodium taurocholate co-transporting polypeptide; pgRNA, pre-genomic RNA; rcDNA, relaxed circular DNA. *Detectable in the bloodstream.

**Abbreviations:**
CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; WHO, World Health Organization; HBV, hepatitis B virus; DNA, double-stranded deoxy-ribonucleic acid; rcDNA, relaxed circular DNA; NTCP, sodium taurocholate co-transporting polypeptide; cccDNA, covalently closed circular DNA; dslDNA, double-stranded linear DNA; HBV, hepatitis B virus; HBcAg, hepatitis core antigen; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBx, x protein; mRNA, messenger RNA; pgRNA, pre-genomic RNA; rcDNA, relaxed circular DNA; NUCs, nucleos(t)ide analogues; PEG-IFNa, pegylated interferon alpha; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; RACE, rapid amplification of complimentary DNA ends; SVP, subviral particles; qHBSAg, quantitative hepatitis B surface antigen; HBcrAg, hepatitis B core related antigen; P22Cr, precore protein; RDT, rapid diagnostic test; EOT, end-of-therapy; RNAi, RNA interference; siRNAs, small interfering RNAs; ASO, antisense oligonucleotide; CpAM, core protein allosteric modulator
oped and released as infectious virions. The encapsidated rcDNA can be redirected to the nucleus to replenish the intranuclear cccDNA pool. The persistence of cccDNA pool in the hepatocytes is the primary reason why it is not possible to eradicate the virus. A minority of mature HBV virions contain dsDNA, which are replication-deficient but are capable of host genome integration at sites of chromosomal DNA breaks. These form stable templates for synthesis of HBsAg and HBx, and can become potentially carcinogenic.

**TYPES OF ANTIVIRAL TREATMENT**

There are two types of approved antiviral therapy in CHB: nucleos(t)ide analogues (NUCs) and pegylated interferon-α (PEG-IFNa). NUCs are DNA polymerase inhibitors that target the step of reverse transcription. As only a single step of the viral replication cycle is inhibited, there is relatively limited effects on the upstream events. The degree of viral suppression is limited to DNA synthesis, whereas cccDNA remains largely unaffected, and it would take a long time for the latter to decline. Therefore, NUCs need to be taken on a long-term basis, as premature withdrawal is associated with high rates of virological rebound. The current first-line NUCs include entecavir, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide, all of which have a high barrier to viral resistance, and are generally well-tolerated.

The mechanisms of action for PEG-IFNa are less well-defined, but is believed to exert both immunomodulatory functions and direct antiviral properties. IFNα treatment induces a non-cytolytic antiviral state in the hepatocytes via regulation of gene expression and protein translation. One of the key mechanisms involve upregulation of APOBEC3 (a cytidine deaminase) which induces G-to-A hypermutations in the HBV genome and thereby inhibits viral replication or even cccDNA degradation. Also, IFNα treatment leads to cccDNA-bound histone hypoacetylation and decreased binding to STAT1/STAT2 transcription factors, leading to reduced transcription of pgRNA from the cccDNA template. Although PEG-IFNa can be given for a finite period (48-week course) as opposed to NUCs, HBV DNA suppression was suboptimal. In addition, it is administered subcutaneously and associated with numerous side effects, rendering it a less utilized treatment option in CHB.

**SERUM VIRAL MARKERS TO EVALUATE TREATMENT RESPONSE**

To assess treatment response, a number of viral biomarkers can be measured in the blood as a surrogate of the ongoing viral replicatory activities (Fig. 1). Well established markers such as HBV DNA and HBsAg have been incorporated as treatment endpoints in the CHB cascade of care (Fig. 2). On-treatment virological suppression, also known as incomplete cure, is the most reachable endpoint and can be achieved in >90% of NUC-treated subjects. Partial cure is defined as off-therapy virological suppression without HBsAg seroclearance, which is observed in around 20% subjects who received a finite course of therapy. Functional cure refers to sustained HBsAg seroclearance plus ≥6 months undetectable HBV DNA, which is associated with improved clinical outcomes but is only achieved by ~1% antiviral-treated subjects annually. Complete cure is defined as eradication of cccDNA, and sterilizing cure is defined as clearance of integrated DNA; both of which are unreachable with the current treatments. With these considerations, functional cure is regarded as the desirable treatment endpoint and has become a benchmark for phase 3 clinical trials of novel CHB therapy, with a threshold of HBsAg loss ≥30% as an arbitrarily acceptable rate of response 6 months after cessation of investigational compounds.

The widespread use of blood-based viral biomarker stems from the need to quantify transcriptionally active intrahepatic cccDNA, which requires tissue specimens obtained from liver biopsy. Due to the invasive nature of the procedure, together with concerns from sampling error, intra/inter-observer variability and lack of standardization of the measurement, cccDNA quantification has largely remained as a research tool. To this end, a number of blood-based HBV biomarkers has been studied as surrogate markers for cccDNA. They can be broadly classified as viral nucleic acids and translational products of HBV.

**Viral nucleic acids**

**HBV DNA**

The vast majority of detectable serum circulating HBV DNA is in the form of enveloped/encapsidated rcDNA. In untreated patients, it shows moderate to good correlation with intrahepatic cccDNA (correlation coefficient r 0.36–0.49).
The widely used **in vitro** nucleic acid amplification method allows high sensitivity of DNA detection and quantification, with lower limits reaching or below 1 to 2 log.

Upon NUC therapy, serum HBV DNA declines rapidly to undetectable levels. When assessed at 48 weeks, first-line NUC leads to undetectable serum HBV DNA in 64–76% and 90–94% of HBeAg-positive and HBeAg-negative patients, respectively. 

For PEG-IFNa, after the complete course of 48 weeks, HBV DNA undetectability can be achieved in only 14% and 19% HBeAg-positive and HBeAg-negative patients, respectively.

HBV **pgRNA**

Circulating HBV RNA are encapsidated pgRNA in virus-like particles. In untreated patients, it shows good to excellent correlation with intrahepatic cccDNA (r=0.59–89). Serum pgRNA can be measured with rapid amplification of complimentary DNA ends-based real-time polymerase chain reaction method, and the performance of RNA assays has been improved recently to approach the WHO standards. Prior to antiviral treatment, serum HBV pgRNA levels are always 1–2 log lower than serum HBV DNA.

After a period of NUC treatment, the serum HBV RNA levels were decreased to a lesser extent than HBV DNA, leading to a reverse in serum RNA:DNA ratio. Like serum HBV DNA, the correlation with cccDNA will be lost after antiviral therapy. The 48-week decline in HBV RNA was 1.46 log upon NUC treatment. When assessed at 48 weeks of PEG-IFNa therapy among HBeAg-positive patients, the mean HBV RNA declined from 7.73 to 4.66 log. For HBeAg-negative patients, upon PEG-IFNa and assessed at 48 weeks of therapy, a 1.72 log decline was observed from a baseline mean level of 4.4 log. Unlike serum HBV DNA, the current use of HBV pgRNA measurement remains in the research context with no widely accepted standard to facilitate implementation in clinical use, and few comparisons of the various assays have been performed so far.

**Translational products**

**HBeAg**

The qualitative HBeAg has been more clinically relevant, being used to stratify disease phase and as an endpoint of treatment among HBeAg-positive patients (i.e., HBeAg seroclearance or seroconversion). In contrast, the quantitative HBeAg levels are mainly for research purpose, which can be...
HBsAg

The majority of HBsAg detected in the serum are subviral particles (SVP), which exceed mature virions by 100–100,000 times. Quantitative HBsAg levels correlated with serum HBV RNA \( r = 0.68 \), DNA \( r = 0.53 \), qHBsAg \( r = 0.20 \) and HBcrAg \( r = 0.69 \). In patients (predominantly genotype B/C) treated with PEG-IFNa, HBsAg levels declined starting from week 12 of therapy only in patients who achieved subsequent HBsAg seroconversion. HBsAg less than 17.55 PEI-U/mL at week 12 had positive predictive value and negative predictive value of 38% and 95% to predict HBsAg seroconversion at week 48. In another study involving CHB patients of Chinese ethnicity, baseline HBsAg levels were incorporated into a risk score which also include other biochemical variables (alanine aminotransferase, globulin and gamma-glutamyl transpeptidase) with a C-index of 0.776 to predict HBsAg seroconversion at 1 year.

HBsAg seroclearance rate at 48 weeks of NUC treatment is 0–1%, although the event rate will slowly increase upon long-term treatment to <2% per year. For PEG-IFNa recipients, the 1-year HBsAg seroclearance rate is 4% and slowly increases with time after treatment completion (2.4% in 6.1 years).

For patients treated with NUCs, the annual decline of qHBsAg was only 0.107 log, with only 16.1% patients achieving ≥1 log decline from baseline at 1 year of TDF therapy. In contrast, PEG-IFNa treatment resulted in a larger magnitude of qHBsAg decline. After 48 weeks of PEG-IFNa treatment, a 0.71 log decline in qHBsAg levels was observed. In addition, treatment responders were likely to have more significant decline in qHBsAg levels during the early phase of treatment. In view of this characteristic, qHBsAg profile (baseline level and on-treatment decline) has been incorporated in treatment algorithms to indicate treatment futility and for consideration of treatment cessation. For HBeAg-positive CHB patients, qHBsAg level >20,000 for genotype B/C or no decline of qHBsAg for genotype A/D at 12 weeks of PEG-IFNa fulfils the treatment-stopping criteria. If the week 24 qHBsAg remains >20,000 IU/L, PEG-IFNa should also be stopped regardless of genotype. Similarly, for HBeAg-negative CHB patients with genotype D infection, absence of qHBsAg decline in combination with <2 log reduction in serum HBV DNA at 12 weeks should also be regarded as futile.

HBcrAg

Hepatitis B core-related antigen (HBcrAg) is a composite of 3 related proteins that share an identical 149 amino acid sequence: HBcAg, HBeAg and a truncated 22 kDa precore protein (p22Cr) that is a processed product of the precore protein; see Figure 1. The chemiluminescence signal is generated from immunocomplexes formed between HBcrAg and alkaline phosphatase-labelled anti-HBcrAg antibodies, after which the quantity can be derived from known concentrations of recombinant ProHBcAg. HBcrAg demonstrates good correlation with intrahepatic cccDNA \( r = 0.48–0.70 \) in both untreated and NUC-treated subjects.

Measurement of HBcrAg at 48 weeks of first-line NUCs or 52 weeks of PEG-IFNa therapy demonstrated a median decline of 1.37 log. Reduction in HBcrAg was correlated with reduction in cccDNA \( r = 0.503 \). The main limitation with HBcrAg is the relatively high lower limit of detection (3 log UI/mL), and is not detectable in up to 30% of HBeAg-negative patients. A recent novel HBcrAg assay demonstrated an improved sensitivity of 2.1 log U/mL, and potentially will provide more insights in the viral kinetics and changes upon treatment especially in HBeAg-negative patients.

Table 1 summarizes the treatment effects on hepatitis B viral biomarkers at 1 year stratified by treatment type.

POTENTIAL APPLICATION OF VIRAL MARKERS

Blood-based HBV biomarkers are crucial for evaluating treatment candidacy, treatment response in both approved therapies and novel drugs in the pipeline.

Decision on treatment candidacy

Not all CHB subjects are eligible for antiviral treatment. In the various clinical guidelines, serum viral biomarkers are essential to determine treatment candidacy. Serum HBV DNA remains the most important parameter, although qualitative HBeAg is included in the American Association for the Study of Liver Diseases (AASLD) and APASL guidelines to decide on the threshold of HBV DNA above which treatment is indicated. In general, serum HBV DNA >20,000 IU/mL (for HBeAg-positive subjects) or >2,000 IU/mL (for HBeAg-negative subjects) plus elevated serum alanine aminotransferase or presence of other risk features would be considered eligible for treatment. In cirrhotic patients, the HBV DNA threshold for treatment would be much lowered.

Higher serum HBcrAg were independently associated with immune tolerance over immune clearance among HBeAg-positive patients (8.2 vs 7.6 log). In contrast, lower serum qHBsAg levels were independently associated with inactive carrier state and HBsAg seroclearance. These biomarkers might play a role to identify patients requiring antiviral therapy in the HBeAg-positive and HBeAg-negative phase, respectively.

In the setting of prevention of mother-to-child-transmission, the WHO recommends HBV DNA testing to decide whether antiviral prophylaxis should be given during pregnancy. HBV DNA >200,000 IU/mL is regarded the threshold to initiate TDF treatment. Where antenatal HBV DNA testing is unavailable, both HBeAg (qualitative) and qHBsAg can be used as a surrogate marker to determine eligibility of TDF prophylaxis. HBeAg positivity has a sensitivity of 88.2% and specificity of 92.6% to detect HBV DNA >200,000 IU/mL. Likewise, serum qHBsAg >4 log is 85.1% sensitive and 96.5% specific for HBV DNA >200,000 IU/mL.

A recently developed Xpert® HBV Viral Load assay for HBV DNA has shown promise to accurately quantify HBV DNA in dried blood spots, with 85.4% having estimable viral loads to within 1 log of the corresponding serum load, with a limit of detection of 7.5 IU/mL. This approach would be very helpful in many resource-limited settings especially where the GeneXpert® system is already in place for the purpose of analysing other pathogens such as SARS-CoV-2 or Mycobacterium tuberculosis & rifampin resistance. Another point-of-care rapid diagnostic test (RDT) for HBcrAg has recently been developed using stored sera as a simplified assessment tool especially in settings where HBV DNA or qHBsAg are not routinely available. With a detection limit of 4.3 log U/mL, the RDT-HBcrAg can identify highly viremic patients that fulfil treatment criteria according to clinical guidelines, with sensitivity 90.5–96.6% and specificity 83.2–96.8%. The RDT-HBcrAg kit has a low production cost (<USD 5), reasonable operating temperature (18–39°C) with simple sample handling without needing any specific equipment or molecular laboratory facilities. More validation studies for this kit as well as the cost-effectiveness of this approach in resource-limited settings should be evaluated.

Table 1. Summary of treatment effects on hepatitis B viral biomarkers at 48 or 52 weeks according to treatment type

<table>
<thead>
<tr>
<th>Assessment criteria</th>
<th>First-line NUCs</th>
<th>PEG-IFNα</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA undetectability</td>
<td>HBeAg-positive: 64–76%</td>
<td>HBeAg-positive: 14%</td>
</tr>
<tr>
<td></td>
<td>HBeAg-negative: 90–94%</td>
<td>HBeAg-negative: 19%</td>
</tr>
<tr>
<td>HBV RNA</td>
<td>-1.46 log</td>
<td>HBeAg-positive: -7.73 to -4.66 log</td>
</tr>
<tr>
<td></td>
<td>HBeAg-negative: -1.72 log</td>
<td></td>
</tr>
<tr>
<td>HBeAg seroclearance</td>
<td>HBeAg-positive: 10–21%</td>
<td>HBeAg-positive: 32%</td>
</tr>
<tr>
<td>HBsAg seroclearance</td>
<td>&lt;1%</td>
<td>4%</td>
</tr>
<tr>
<td>qHBsAg</td>
<td>-0.107 log (average rate per year)</td>
<td>-0.71 log</td>
</tr>
<tr>
<td>HBcrAg</td>
<td>-1.37 log</td>
<td>-1.37 log</td>
</tr>
</tbody>
</table>

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NUCs, nucleos(t)ide analogues; PEG-IFNα, pegylated interferon alpha; qHBsAg, quantitative hepatitis B surface antigen.
Dose adjustment and regimen modification

With the understanding of the on-treatment profile of HBV DNA and qHBsAg, both are essential markers to be monitored during the course of therapy. In NUC recipients, HBV DNA monitoring is essential to detect virological breakthrough which would suggest either primary resistance or non-compliance to treatment. In PEG-IFNa recipients, stopping rules are defined according to qHBsAg levels as discussed above.

Risk stratification for HCC

Viral biomarkers give important clues in the risk of HCC among treated CHB subjects. Serum qHBsAg has been shown to be associated with HCC risk. The hazard ratio for developing HCC was 13.7 for low viremic (HBV DNA <2,000 IU/mL) HBeAg-negative patients with serum qHBsAg ≥3 log compared to those with serum qHBsAg <3 log. Moreover, HBsAg seroclearance, i.e., functional cure, is associated with significantly reduced HCC risk, especially in subjects who achieved this endpoint before the age of 50 and regardless of whether the patient was given antiviral therapy. Serum viral load (HBV DNA) is a well-known risk factor for HCC and demonstrated a biological gradient in the REVEAL-HBV cohort. Long term NUC treatment has been shown to reduce the risk of HCC. Since HBV DNA is no longer detectable in the serum (in the majority of cases) upon NUC treatment, other viral biomarkers have been explored to assess the risk of HCC in antiviral-treated CHB patients. In this context, serum HBcrAg and pgRNA might aid risk stratification in addition to serum HBV DNA and qHBsAg levels. While serum HBcrAg is reduced in all NUC-treated CHB patients, a high post-treatment HBcrAg was associated with >2 fold increase in risk of HCC. Similarly, on-treatment detectable serum pgRNA is associated with 3.5-fold higher risk of HCC in 2 years’ time.

Prediction of partial/functional cure

Among HBeAg-positive patients, a higher baseline serum HBcrAg was independently associated with NA-induced HBeAg seroconversion, while a lower HBcrAg at week 12 of PEG-IFN was predictive of HBeAg seroclearance and HBV DNA <2,000 IU/mL at 24 weeks post-treatment. HBeAg seroclearance/seroconversion is the pre-requisite for cessation of long-term NUC among HBeAg-positive patients, after HBV DNA undetectability for a certain period, in order to achieve incomplete cure. Numerous studies have explored the success rate and predictors for off-therapy virological control. Apart from host factors, viral factors might provide insights in risk of virological or clinical relapse after stopping long term NUC. Low end-of-therapy (EOT) serum qHBsAg, preferably <100 IU/mL, has been consistently shown to predict partial cure. In addition, low EOT serum HBcrAg, undetectable EOT serum HBV pgRNA, or a combination of both, identified a subgroup of patients who would be able to stop long-term NUC with a lower chance of flare. Some patients with a favourable viral biomarker profile would benefit from such approach and achieve functional cure. In fact, assessing viral biomarkers (serum HBcrAg and pgRNA) as early as week 4 of NUC treatment is able to highlight a group of patients who would achieve a low serum qHBsAg (<100 IU/mL) or HBsAg seroclearance in the long run. This approach can help to identify subjects during the early phase who should not stop NUC and should be prioritized into clinical trials.

Evaluation of efficacy and target engagement for novel compounds

The treatment landscape of CHB is expected to change with the numerous novel agents being explored; detailed discussion of these therapeutic approaches has been reviewed elsewhere. These drugs target alternative steps in the viral replication cycle, stimulate host immune response, or act on both pathways. As mentioned above, functional cure is the desirable treatment endpoint for phase 3 clinical trials of novel CHB therapy.

At the time of writing, several novel compounds have demonstrated promising results on sustainable HBsAg suppression. RNA interference-based therapy with either small interfering RNAs or antisense oligonucleotide (ASO) were able to knock down HBsAg levels by more than 1 log within <48 weeks of treatment. For instance, JNJ-3989, a siRNA, when given with NUC led to HBsAg reduction by ≥1 log from baseline in 39/40 (97.5%) subjects at nadir, which persisted in 38% patients at 1 year post EOT. The mean declines of HBeAg, HBcrAg and HBV RNA from baseline to 16 weeks were 1.47 log, 1.2 log and 1.93 log respectively.
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Decision on treatment candidacy</th>
<th>Dose adjustment or regimen modification</th>
<th>Risk stratification for HCC</th>
<th>Prediction of partial cure or functional cure</th>
<th>Evaluation of target engagement for novel compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA</td>
<td>Highly viremic: indicated for treatment</td>
<td>NUC viral resistance: switch to another class of NUC</td>
<td>Residual viraemia increases risk of HCC</td>
<td>Undetectable HBV DNA for a period of consolidation is pre-requisite for NUC cessation</td>
<td>RNAi-based therapy/CpAM</td>
</tr>
<tr>
<td>HBV RNA</td>
<td>-</td>
<td>-</td>
<td>Residual viraemia increases risk of HCC</td>
<td>Lower levels predict partial cure and functional cure</td>
<td>RNAi-based therapy/CpAM</td>
</tr>
<tr>
<td>HBeAg (qualitative)</td>
<td>Prevention of MTCT</td>
<td>PEG-IFNa: HBeAg seroclearance is the treatment endpoint for HBeAg-positive patients</td>
<td>-</td>
<td>HBeAg seroclearance is pre-requisite for NUC cessation</td>
<td>RNAi-based therapy</td>
</tr>
<tr>
<td>HBsAg (qualitative)</td>
<td>-</td>
<td>HBsAg seroclearance is associated with reduced risk of HCC</td>
<td>Defines functional cure</td>
<td>Undetectable serum HBsAg is the primary endpoint for phase III trials</td>
<td></td>
</tr>
<tr>
<td>qHBsAg</td>
<td>Prevention of MTCT</td>
<td>PEG-IFNa: stopping rule</td>
<td>Predict risk of HCC in low viremic patients</td>
<td>Lower levels predict partial cure and functional cure</td>
<td>RNAi-based therapy</td>
</tr>
<tr>
<td>HBcrAg</td>
<td>RDT point of care test for identifying highly viremic patients</td>
<td>Predict immune tolerance in HBeAg(+) patients</td>
<td>Higher on-treatment levels increase risk of HCC</td>
<td>Lower levels predict partial cure and functional cure Predict response to NA or PEG-IFN</td>
<td>RNAi-based therapy/CpAM</td>
</tr>
</tbody>
</table>

CpAM, core protein allosteric modulator; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; MTCT, mother-to-child-transmission; NUCs, nucleos(t)ide analogues; PEG-IFNa, pegylated interferon alpha; qHBsAg, quantitative hepatitis B surface antigen; RDT, rapid diagnostic test; RNAi, RNA interference.
9–10% participants assessed at 24 weeks post-EOT. However, despite the large number of ongoing trials, no compounds have reached the benchmark of inducing functional cure in ≥30% subjects. It is therefore important to understand the mechanisms of action for various novel compounds and utilize the appropriate viral biomarkers to evaluate target engagement as an interim response. Core protein allosteric modulator (CpAM) inhibits the formation of functional capsids and encapsidation, thereby reducing the amount of circulating encapsidated pgRNA. In patients who received vebicorvir (CpAM), significant reductions in serum HBV DNA and pgRNA were observed at week 12 and 24 even though no change in serum qHBsAg was seen. The inhibition of pgRNA synthesis could be observed as early as day 15 in patients receiving ABI-H2158 (CpAM), with mean decline of >2 log from baseline compared to 0.03 log in the placebo group. Other viral markers such as HBeAg and HBcrAg levels were evaluated in some of the trials involving CpAM. According to a recent study with treatment-naive cohorts receiving 48 weeks of NUCs+RO7049389 (CpAM) or NUCs+RO7049389+PEG-IFNa, the mean declines of HBeAg were 1.48 and 2.10 log IU/mL and for HBcrAg 1.23 and 1.76 log U/mL respectively. However, the long-term durability of the viral kinetic changes during novel therapies, as well as predictive factors for a durable suppression of various viral biomarkers, remains largely unclear and should be evaluated in future clinical trials.

Table 2 summarizes the potential applications of the viral biomarkers discussed in various settings.

**CONCLUSION**

Viral biomarker assessment is indispensable in clinical management and through the journey of novel drug discovery in the field of CHB. In the current era with highly effective NUC therapy as the mainstay of treatment, HBV DNA will be expectedly undetectable and novel transcriptional (HBV RNA) and translational markers (qHBsAg and HBcrAg) can provide further insights into treatment efficacy. Emerging data suggests these viral biomarkers can aid treatment decision, risk stratification for HCC and risk prediction for partial cure/functional cure. As the primary outcome of phase III trials is set on functional cure, viral biomarkers can potentially inform the efficacy of novel compounds or treatment approaches in the early course of treatment, and help with prioritization of subjects into clinical trials. Importantly, standardization and validation studies are necessary before viral biomarkers can be broadly implemented in clinical use. The role of viral biomarkers needs to be further explored to pave the way into elimination of viral hepatitis B.

**Authors’ contribution**

LYM was responsible literature search, critical appraisal and drafting of the manuscript. RWHH, JF and WKS were responsible for critical revision of the article. MFY was responsible for conception of the work and critical approval of the article.

**Conflicts of Interest**

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

**REFERENCES**

272

Clinical and Molecular Hepatology
Volume_29 Number_2 April 2023


http://www.e-cmh.org

272

https://doi.org/10.3350/cmh.2022.0448


36. Ma H, Yang RF, Wei L. Quantitative serum HBsAg and HBeAg are strong predictors of sustained HBeAg serocconversion to pegylated interferon alfa-2b in HBeAg-positive patients. J Gastroenterol Hepatol 2010;25:1498-1506.


40. Roche. Elecsys® HBsAg II: Immunoassay for the qualitative determination of hepatitis B surface antigen (HBsAg).


64. Lee HA, Lee HW, Park Y, Kim HS, Seo YS. Hepatitis B core-related antigen is useful for predicting phase and prognosis of hepatitis B e antigen-positive patients. J Clin Med 2022;11:1729.


Utility of combining PIVKA-II and AFP in the surveillance and monitoring of hepatocellular carcinoma in the Asia-Pacific region


Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; Clinical Pathology Department, Medic Center, Ho Chi Minh, Vietnam; Department of Gastroenterology and Hepatology, Singapore General Hospital, Singapore; Faculty of Medicine, University Indonesia/Ciptomangunkusumo Hospital, Jakarta, Indonesia; Clinical Pathology Department, Integrated Laboratory, Dharmais National Cancer Hospital, Jakarta, Indonesia; Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital; College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; School of Medicine, College of Medicine and Center of Excellence for Metabolic Associated Fatty Liver Disease, National Sun Yat-sen University, Kaohsiung, Taiwan; Japanese Red Cross Musashino Hospital, Musashino, Japan; National Hospital for Tropical Diseases, Hanoi, Vietnam; Department of Hepato-pancreato-biliary and Transplant Surgery, National Cancer Center Singapore and Singapore General Hospital, Singapore; Surgery Academic Clinical Program, Duke-NUS Medical School, Singapore; University Malaya Medical Centre, Kuala Lumpur, Malaysia; State Key Laboratory of Oncology in South China, Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong; Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; Department of Medicine, School of Medicine, Chung Shan Medical University, Taichung, Taiwan; Biochemistry Department, Hanoi Medical University, Hanoi, Vietnam; Eastern Hepatobiliary Surgery Hospital, Second Military Medical University [Navy Medical University], Shanghai, China; Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Department of Internal Medicine, The Chinese University of Hong Kong and Union Hospital, Hong Kong

Even though the combined use of ultrasound (US) and alpha-fetoprotein (AFP) is recommended for the surveillance of hepatocellular carcinoma (HCC), the utilization of AFP has its challenges, including accuracy dependent on its cut-off levels, degree of liver necroinflammation, and etiology of liver disease. Though various studies have demonstrated the utility of protein induced by vitamin K absence II (PIVKA-II) in surveillance, treatment monitoring, and predicting recurrence, it is still not recommended as a routine biomarker test. A panel of 17 experts from Asia-Pacific, gathered to discuss and reach a consensus on the clinical usefulness and value of PIVKA-II for the surveillance and treatment monitoring of HCC, based on six predetermined statements. The experts agreed that PIVKA-II was valuable in the detection of HCC in AFP-negative patients, and could potentially benefit detection of early HCC in combination with AFP. PIVKA-II is clinically useful for monitoring curative and intra-arterial locoregional treatments, outcomes, and recurrence, and could potentially predict microvascular invasion risk and facilitate patient selection for liver transplant. However, combining PIVKA-II with US and AFP for HCC surveillance, including small HCC, still requires more evidence, whilst its role in detecting AFP-negative HCC will potentially increase as more patients are treated for hepatitis-related HCC. PIVKA-II in combination with AFP and US has a clinical role in the Asia-Pacific region for surveillance. However, implementation of PIVKA-II in the region will have some challenges, such as requiring standardization of cut-off values, its cost-effectiveness and improving awareness among healthcare providers. (Clin Mol Hepatol 2023;29:277-292)

Keywords: PIVKA-II; Alpha-fetoprotein; Carcinoma, hepatocellular; Consensus; Biomarkers
INTRODUCTION

With an estimated 60.0% increase by 2040, hepatocellular carcinoma (HCC) remains a global disease burden. An estimated 85.0% of HCC patients are in low- and middle-resource countries, with Asia carrying the largest burden of >20 cases per 100,000 population. Beyond the exposure to risk factors, the incidence and mortality rates of HCC are closely associated with the availability of healthcare resources for detecting early-stage disease, and access to potential curative treatment.

The Asian Pacific Association for the Study of the Liver (APASL) guidelines recommend biannual surveillance using a combination of ultrasound (US) and alpha-fetoprotein (AFP) in all high-risk individuals for the early detection of HCC, in order to improve the survival rate of HCC patients.

AFP has had an established role as a biomarker in HCC for decades. It is a standardised test considered more objective than imaging alone, and is easily accessible. Its optimal utility in surveillance is in combination with US, and is useful for confirming inconclusive imaging results. However, even in combination with US, AFP has its challenges, including sensitivity and specificity, which are dependent on various factors. These include the cut-off levels used, the degree of necroinflammation of the liver, and the aetiology of the liver disease. In addition, up to 80.0% of small HCC (tumour size ≤3 cm) and early-stage HCC tumours are not picked up by AFP. Protein induced by vitamin K absence II (PIVKA-II), also known as Des-γ-carboxy (abnormal) prothrombin (DCP), was first described in 1968. In 1984, it was detected in 90.0% of patients with HCC, suggesting that it could have a potential use as an HCC biomarker. Though there are multiple studies on its utility in surveillance, treatment monitoring, and predicting recurrence of HCC, it is not yet recommended as a routine test.

The objective of this consensus paper is to discuss the clinical usefulness and value of PIVKA-II in the Asia-Pacific region for the surveillance and treatment monitoring of HCC, its benefits and limitations, and further steps required to improve its utility.

METHODS

A group of 17 experts in hepatology, surgical oncology, medical oncology, and laboratory medicine (Table 1) from countries across Asia-Pacific, was identified to develop this consensus statement that would define the clinical utility of PIVKA-II in the surveillance and treatment monitoring of HCC in the region. The experts convened via an online meeting to share the latest relevant available evidence on PIVKA-II, and to vote on predetermined statements (R1). The votes were to agree or disagree with each statement based on the evidence and the expert’s opinions. Statements that were disagreed on were discussed, and the experts’ points taken into consideration for refinement.

The second (R2) and third (R3) rounds of voting were conducted by emailing each expert the reworded statements presented with the same binary (agree/disagree) options and an open-ended remark column in case of disagreement. After each round, the comments of individual experts were considered and the statements edited further. The final state-
ments, with their agreement, were used to develop the first draft. The agreement for each statement follows a 3-point scale of "inconclusive", "agree with condition", and "strongly agree", based on the proportion of experts agreeing on it (Table 2).

A web review of the draft consensus statement was done to gather feedback from all experts. It included instructions for a final round of voting (R4) on the statements, and a review of the discussion and evidence presented for each statement. All comments were reviewed by the chairpersons, and the manuscript edited. A final round of review was performed by the experts before the manuscript was finalised.

**FINDINGS**

The final consensus statements, the agreement reached (Table 3), and a summary of the evidence for each, are presented here. For discussions on the implementation of PIVKA-II, the experts’ opinions are presented.

**The role of PIVKA-II in HCC surveillance**

Statement 1: PIVKA-II in combination with AFP improves the detection of HCC, including small sized tumours (≤3 cm), compared to either biomarker alone

Agreement: Strongly agree

Although PVIKA-II alone has shown adequate accuracy in detecting HCC, combining the test with AFP results in better

---

**Table 1. Expert panel**

<table>
<thead>
<tr>
<th>Name</th>
<th>Specialty</th>
<th>Country/Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do-Young Kim (Co-Chair)</td>
<td>Hepatology</td>
<td>South Korea</td>
</tr>
<tr>
<td>Henry Chan (Co-Chair)</td>
<td>Hepatology</td>
<td>Hong Kong, China</td>
</tr>
<tr>
<td>Irsan Hasan</td>
<td>Hepatology</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Namiki Izumi</td>
<td>Hepatology</td>
<td>Japan</td>
</tr>
<tr>
<td>Chee-Kiat Tan</td>
<td>Hepatology</td>
<td>Singapore</td>
</tr>
<tr>
<td>Ming-Lung Yu</td>
<td>Hepatology</td>
<td>Taiwan</td>
</tr>
<tr>
<td>Teng-Yu Lee</td>
<td>Hepatology</td>
<td>Taiwan</td>
</tr>
<tr>
<td>Tawesak Tanwandee</td>
<td>Hepatology</td>
<td>Thailand</td>
</tr>
<tr>
<td>Nguyen Nguyen Huyen</td>
<td>Hepatology</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Rosmawati Mohamed</td>
<td>Hepatology</td>
<td>Malaysia</td>
</tr>
<tr>
<td>Lyana Setiawan</td>
<td>Laboratory medicine</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Woo-Chang Lee</td>
<td>Laboratory medicine</td>
<td>South Korea</td>
</tr>
<tr>
<td>Bao Nguyen Toan</td>
<td>Laboratory medicine</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Thi Thanh Nguyen Hai</td>
<td>Laboratory medicine</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Tian Yang</td>
<td>Hepatobiliary surgery</td>
<td>China</td>
</tr>
<tr>
<td>Pierce Chow</td>
<td>Hepatobiliary surgery</td>
<td>Singapore</td>
</tr>
<tr>
<td>Stephen Chan</td>
<td>Medical oncology</td>
<td>Hong Kong, China</td>
</tr>
</tbody>
</table>

The expert panel is listed based on specialty, and alphabetically arranged according to country/area, except for the chairpersons.

**Table 2. The agreement scale used for the consensus statements**

<table>
<thead>
<tr>
<th>Agreement</th>
<th>Proportion voting on “Agree”</th>
<th>Number of experts who voted “Agree” (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inconclusive</td>
<td>&lt;50.0%</td>
<td>≤8</td>
</tr>
<tr>
<td>Agree with condition</td>
<td>50.0–80.0%</td>
<td>9–14</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>&gt;80.0%</td>
<td>≥15</td>
</tr>
</tbody>
</table>

The agreement accompanying each consensus statement is based on the final round of voting (R4).
surveillance (Table 4) across the high-risk groups, as it combines their individual benefits. However, the variable accuracy of both tests, depending on the cut-off values used, must be considered when interpreting the results. The data from studies suggest that the optimal cut-off value for PIVKA-II when used in combination with AFP is 40 mAU/ml but further validation is required.\(^9\)\(^-\)\(^14\)

Among the studies conducted to determine the accuracy of PIVKA-II alone, AFP alone and combining both biomarkers, only a few meet the optimal level of evidence as described by Early Detection of Research Network (EDRN)\(^1\)\(^5\) and the International Liver Cancer Association.\(^6\) Lok et al.\(^1\)\(^3\) compared the accuracy of AFP and PIVKA-II in the early diagnosis of HCC in a nested-control study within the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial.\(^1\)\(^7\) The results demonstrated that combining both biomarkers increased the sensitivity but decreased the specificity of the individual biomarkers to detect early HCC. The sensitivity increased to 91.0% at the time of diagnosis and 73.0%, 12 months prior to diagnosis, and the specificity reduced to 74.0% and 71.0%, at the two time-points respectively.\(^1\)\(^3\) The three other EDRN phase 3 biomarker studies\(^1\)\(^8\)\(^-\)\(^1\)\(^0\) to determine accuracy of biomarkers for surveillance of early HCC included an additional biomarker, i.e., lectin-reactive AFP alone or within the GALAD (Gender, Age, AFP-L3, AFP and PIVKA-II), and are beyond the scope of this position paper.

A systematic review of 38 studies with 11,124 cases, revealed that PIVKA-II alone was only moderately accurate in detecting HCC (sensitivity 0.66, 95% confidence interval [CI] 0.65–0.68; specificity 0.88, 95% CI 0.87–0.90; positive likelihood ratio (+LR) 7.13, 95% CI 5.73–8.87; negative likelihood ratio (-LR) 0.33, 95% CI 0.29–0.38).\(^2\)\(^1\)

On the other hand, a pooled analysis demonstrated that combining PIVKA-II and AFP improved sensitivity and specificity compared to either test alone (PIVKA-II+AFP, 82.0% and 85.0% vs. AFP alone, 65.0% and 88.0%, and PIVKA-II alone, 69.0% and 89.0%, respectively).\(^1\)\(^3\) The AUC also increased by combining both tests (PIVKA-II+AFP, 0.90 vs. AFP, 0.88 and PIVKA-II, 0.75, respectively). These findings were in line with other studies (Table 5).\(^9\)\(^-\)\(^2\)\(^4\) Similarly, real-world data demonstrated that PIVKA-II (cut-off value at 40 mAU/ml) is a necessary complement to AFP (cut-off value at 20 ng/mL) and US in surveillance.\(^2\)\(^5\)

However, there is a trade-off to be expected with increasing the sensitivity of the tests. Though meta-analyses seeking heterogeneity found that threshold levels do not impact the accuracy of the tests,\(^1\)\(^1\)\(^6\)\(^-\)\(^2\)\(^6\) higher cut-off values for either marker reduced sensitivity while improving specificity.\(^1\)\(^3\) Additionally, the higher-level evidence studies\(^1\)\(^3\)\(^,\)\(^1\)\(^8\) have shown that combination of biomarkers could increase sensitivity, however, could markedly decrease specificity. For surveillance, though specificity of the test has a role, an improved sensitivity is more pertinent so as to rule in cases.

The performance of PIVKA-II and AFP also differ depending on the HCC aetiology\(^1\)\(^3\)\(^,\)\(^1\)\(^3\)\(^,\)\(^2\)\(^3\)\(^,\)\(^2\)\(^7\) The accuracy of AFP and PIVKA-II when analysed in cirrhotic patients with chronic liver disease (n=388) demonstrated that both biomarkers’ performances were significantly influenced by the aetiology and activity of the chronic liver disease (Table 4).\(^2\)\(^4\)

### Table 3. Key statements and agreements reached (n=17)

<table>
<thead>
<tr>
<th>Key statement</th>
<th>Agreement</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIVKA-II in combination with AFP improves the detection of HCC, including small-sized tumours (≤3 cm), compared to either biomarker alone</td>
<td>Strongly agree</td>
<td>88.2%</td>
</tr>
<tr>
<td>PIVKA-II is valuable in the detection of HCC in AFP-negative HCC patients</td>
<td>Strongly agree</td>
<td>100%</td>
</tr>
<tr>
<td>Preoperative PIVKA-II measurement predicts the MVI risk, which may be useful in the assessment of tumour prognosis</td>
<td>Strongly agree</td>
<td>94.1%</td>
</tr>
<tr>
<td>PIVKA-II measurements, before and after curative treatment (resection and RFA), are useful for monitoring treatment outcomes and recurrence</td>
<td>Strongly agree</td>
<td>100%</td>
</tr>
<tr>
<td>PIVKA-II measurements, before and after intra-arterial treatment (TACE and TARE), are clinically useful to indicate response</td>
<td>Strongly agree</td>
<td>94.1%</td>
</tr>
<tr>
<td>Pre-liver transplant PIVKA-II levels are associated with the risk of post-operative HCC recurrence, potentially facilitating patient selection</td>
<td>Strongly agree</td>
<td>88.2%</td>
</tr>
</tbody>
</table>

AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; MVI, microvascular invasion; PIVKA-II, protein induced by vitamin K absence II; RFA, radio-frequency ablation; TACE, transhepatic arterial chemoembolization; TARE, transhepatic arterial radioembolization.
Utility of PIVKA-II and AFP in small HCC

The insidious nature of HCC means that by the time patients are diagnosed, most have very poor outcomes, even for 1-year survival. However, it can be cured by surgical resection, orthotopic liver transplantation, or local ablation, if diagnosed early. Small HCC, with nodules of <3 cm, indicates early HCC, and patients with tumours of ≤2 cm have a 5-year survival rate of close to 100%. Hence, the early diagnosis of HCC is essential to improve outcomes for patients.

The recommended method of surveillance (US+AFP) can miss up to 1 in 3 patients with HCC, and adding PIVKA-II could improve the detection of early/small HCC. The accuracy of PIVKA-II and AFP levels alone in diagnosing small HCC is still inconclusive, i.e., results showing either one as being more accurate in terms of sensitivity, specificity, and/or AUC. Combining both markers with cut-off levels maximised for sensitivity and specificity indicates an improvement in the detection of small HCC (Table 5). This suggests that combining AFP and PIVKA-II could be useful in picking up HCC where utilising either marker alone might not.

An important criterion for tests in HCC surveillance is their ability to differentiate between early HCC and other liver diseases like cirrhosis. At cut-off values of 40 mAU/mL for PIVKA-II and 20 ng/mL for AFP, Ji et al. demonstrated that combining both markers improved sensitivity in differentiating small HCC from disease controls compared to either marker alone, but is dependent on the disease type (Table 5).

The challenge is that the cut-off values for each biomarker used in the combination is still inconclusive. In a systematic review of 17 studies, lower cut-off values of PIVKA-II and AFP appear to have had a better overall accuracy than higher cut-off values. However, the diagnostic odds ratio for the higher cut-off values was 2.4 times better than the lower values (59.8 vs. 25.5, respectively). From the analyses, the authors concluded that the optimal cut-off value was 40 mAU/mL for PIVKA-II and 200 ng/mL for AFP. In another study involving 1,361 HCC patients, of which 61.0% (n=834) had small HCC (<3 cm), PIVKA-II 40 mAU/mL and AFP 20 ng/mL together resulted in a sensitivity of 72.0% and specificity of 91.0%.

Statement 2: PIVKA-II is valuable in the detection of HCC in AFP-negative HCC patients

Agreement: Strongly agree

Unlike AFP-positive HCC, AFP-negative HCC (defined as AFP ≤20 ng/mL) are not easily diagnosed, as most present as early or small HCCs. Additionally, the presence of hepatic nodules that resemble HCC tumours on imaging can lead to misdiagnosis. In a large multicentre study, 1,158 patients with HCC were categorised based on AFP levels. The significant proportion of patients had hepatitis B-, hepatitis C- and alcoholic liver disease-related HCC, either alone or in combination. Almost half (46.0%) had normal (<20 ng/mL) AFP levels and only 6.0% (n=66) had AFP levels between 200–400 ng/mL. There is also evidence to suggest a high prevalence of AFP-negative HCC in patients with fatty liver disease, both alcoholic and non-alcoholic.

PIVKA-II, on the other hand, has demonstrated the potential utility in improving the detection of early HCC in AFP-negative HCC patients up to 76.0% (Table 6), though most of the studies involved patients with Hepatitis B virus (HBV)-related aetiology.
<table>
<thead>
<tr>
<th>Author and year</th>
<th>Study type</th>
<th>Number of patients</th>
<th>Cut-off values*</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detection of HCC vs other liver disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guan et al.²⁶ (2022)</td>
<td>Retrospective observational study</td>
<td>n=484 NAFLD (139 NAFLD-HCC, 345 NAFLD control)</td>
<td>40 mAU/mL 20 ng/mL</td>
<td>Non-cirrhotic NAFLD-HCC AUC 0.88 Cirrhotic NAFLD-HCC AUC 0.95</td>
</tr>
<tr>
<td>Seo et al.⁹ (2015)</td>
<td>Retrospective study</td>
<td>n=1,255 CHB (879 non-cirrhotic CHB, 219 LC without HCC, 157 HCC)</td>
<td>40 mAU/mL 10 ng/mL (HCC vs. CHB) 25 ng/mL (HCC vs. LC)</td>
<td>HCC vs. CHB Sn: 75.2% Sp: 95.4% HCC vs. LC Sn: 75.2% Sp: 92.7%</td>
</tr>
<tr>
<td>Chen et al.¹¹ (2017)</td>
<td>Systematic review†</td>
<td>n=27 studies (7,507 HCC patients, 5,399 controls)</td>
<td>Variable</td>
<td>Pooled Sn: 82.0% Pooled Sp: 85.0% AUC: 0.90</td>
</tr>
<tr>
<td>Caviglia et al.²² (2018)</td>
<td>Meta-analysis‡</td>
<td>n=11 studies (873 HCC, 683 LC, 561 CLD)</td>
<td>Variable</td>
<td>sAUC: 0.86</td>
</tr>
<tr>
<td>Ricco et al.²⁴ (2018)</td>
<td>Retrospective study with consecutive sampling§</td>
<td>n=388 (258 LC with HCC, 130 LC without HCC)</td>
<td>48 mAU/mL 20 ng/mL</td>
<td>HCC vs. HS Sn: 80.7% Sp: 67.3%</td>
</tr>
<tr>
<td>Feng et al.²³ (2021)</td>
<td>Prospective study with convenient sampling</td>
<td>n=471 (168 HCC, 150 BLD, 153 HS)</td>
<td>HCC vs. HS 35.6 mAU/mL 17.76 ng/mL HCC vs. BLD 43.47 mAU/mL 21.47 ng/mL</td>
<td>HCC vs. HS Sn: 87.5% Sp: 92.5% AUC: 0.94 HCC vs. BLD Sn: 82.0% Sp: 89.3% AUC: 0.90</td>
</tr>
<tr>
<td><strong>Detection of small HCC (&lt;3 cm)</strong> §</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakamura et al.²¹ (2006)</td>
<td>Prospective study with consecutive sampling</td>
<td>n=1,361 (834 with small HCC)</td>
<td>40 mAU/mL 20 ng/mL</td>
<td>Sn: 72.0% Sp: 91.0%</td>
</tr>
<tr>
<td>Tateishi et al.²⁷ (2008)</td>
<td>Systematic review</td>
<td>n=17 studies</td>
<td>16 mAU/mL 20 ng/mL 40 mAU/mL 200 ng/mL</td>
<td>Sn: 83.0% Sp: 84.0% Sn: 48.0% Sp: 99.0%</td>
</tr>
</tbody>
</table>
### Table 5. Continued

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Study type</th>
<th>Number of patients</th>
<th>Cut-off values*</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrero et al. (2009)</td>
<td>Phase II case-control study</td>
<td>n=836 (417 LC without HCC, 208 early HCC, 211 intermediate/advanced HCC)</td>
<td>150 mAU/mL 20 ng/mL</td>
<td>Sn: 78.0%</td>
</tr>
<tr>
<td>Lok et al. (2010)</td>
<td>Nested case-control study within a phase III randomised controlled trial</td>
<td>n=39 (24 early HCC)</td>
<td>40 mAU/mL 20 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Ji et al. (2016)</td>
<td>A multicentre validation study</td>
<td>n=1,034 (Cohort for differential diagnosis – 236 HCC, 29 MT, 75 LC, 31 LH, 150 HS; Cohort for high-risk patients – 200 HCC, 41 LC, 56 CHB, 150 HS)</td>
<td>40 mAU/mL 20 ng/mL</td>
<td>HCC vs. MT, LC, LH Sn: 90.3% Sp: 66.7%</td>
</tr>
<tr>
<td>Xu et al. (2021)</td>
<td>Retrospective study</td>
<td>n=428 (308 HCC, 60 HBV-related LC, 60 BLD)</td>
<td>40 mAU/mL 25 ng/mL</td>
<td>HCC vs. all control Sn: 83.3% Sp: 89.9% AUC: 0.86</td>
</tr>
</tbody>
</table>

AFP, alpha-fetoprotein; AUC, area under the curve; sAUC, weighted summary area under the curve; BLD, benign liver disease; CHB, chronic hepatitis B; CLD, chronic liver disease; HCC, hepatocellular carcinoma; HS, healthy subjects; LC, liver cirrhosis; LH, liver haemangioma; MT, liver metastasis; PIVKA-II, protein induced by vitamin K absence II; Sn, sensitivity; Sp, specificity.

*Cut-off values in mAU/mL refer to PIVKA-II and in ng/ml refer to AFP. The majority of patients in the control arms had viral hepatitis-related liver disease and different cut-off values were used for PIVKA-II and AFP in each study included in the systematic review or meta-analysis. Multiple cut-off values for PIVKA-II and AFP were tested. The combination that resulted in the best diagnostic accuracy is presented in the table. Small HCC was defined as early HCC, a single tumour nodule of <3cm and no evidence of vascular invasion or metastasis.
<table>
<thead>
<tr>
<th>Author and year</th>
<th>Study type</th>
<th>Number of patients</th>
<th>Cut-off values*</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ji et al. 14 (2016)</td>
<td>A multicentre validation study</td>
<td>n=1,034 (Cohort for differential diagnosis – 236 HCC, 29 MT, 75 LC, 31 LH, 150 HS; Cohort for high-risk patients – 200 HCC, 41 LC, 56 CHB, 150 HS)</td>
<td>40 mAU/mL 20 ng/mL</td>
<td>AFP-negative HCC from all HS Sn: 76.3% Sp: 89.1% AUC: 0.86 AFP-negative HCC from LH, LC and MT Sn: 76.3% Sp: 82.2% AUC: 0.85 AFP-negative HCC from CHB and LC Sn: 63.2% Sp: 90.7% AUC: 0.83</td>
</tr>
<tr>
<td>Xu et al. 34 (2021)</td>
<td>Retrospective study</td>
<td>n=428 (308 HCC, 60 HBV-related LC, 60 BLD)</td>
<td>40 mAU/mL 25 ng/mL</td>
<td>Overall diagnosis of HCC Sn: 83.3% AUC: 0.88</td>
</tr>
<tr>
<td>Wang et al. 25 (2017)</td>
<td>Prospective study consecutive sampling</td>
<td>n=274 (113 early HBV-related HCC, 161 CHB)</td>
<td>32.09 mAU/mL</td>
<td>HCC vs. HS Sn: 51.0% Sp: 84.5%</td>
</tr>
<tr>
<td>Feng et al. 23 (2021)</td>
<td>Prospective study with convenient sampling</td>
<td>n=471 (168 HCC, 150 BLD, 153 HS)</td>
<td>HCC vs. HS 35.6 mAU/mL 17.76 ng/mL HCC vs. BLD 43.47 mAU/mL 21.47 ng/mL</td>
<td>HCC vs. HS Sn: 78.3% Sp: 91.3% AUC: 0.88 HCC vs. BLD Sn: 82.0% Sp: 89.3% AUC: 0.90</td>
</tr>
</tbody>
</table>

AFP, alpha-fetoprotein; AUC, area under the curve; sAUC, weighted summary area under the curve; BLD, benign liver disease; CHB, chronic hepatitis B; CLD, chronic liver disease; HCC, hepatocellular carcinoma; HS, healthy subjects; LC, liver cirrhosis; LH, liver haemangioma; MT, liver metastasis; PIVKA-II, protein induced by vitamin K absence II; Sn, sensitivity; Sp, specificity.

*Cut-off values in mAU/ml refer to PIVKA-II and in ng/mL refer to AFP.
The role of PIVKA-II in prognosis prediction and treatment monitoring

Statement 3: Preoperative PIVKA-II measurement predicts the microvascular invasion (MVI) risk, which may be useful in the assessment of tumour prognosis

Agreement: Strongly agree

Significantly high levels of PIVKA-II based on cut-off values between >40 mAU/mL and >100 mAU/mL appear to predict the occurrence of portal vein tumour thrombosis (PVTT) and MVI, as well as poorer overall survival (OS) and higher risk of recurrence. The challenge in interpreting the data is the wide range of cut-off values used in the studies. Consideration should also be given to the fact that AFP levels and tumour size were also independent predictors for MVI.

A total of 123 newly diagnosed HCC patients (Barcelona Clinic Liver Cancer [BCLC] Stages A–C) were included in a study to determine the correlation of PIVKA-II level to PVTT. PIVKA-II levels were significantly higher in those with PVTT than those without (P=0.003), and had a high area under the receiver operating curve (AUROC) of 0.73, sensitivity of 83.7%, and specificity of 69.2%, at a cut-off of 221.26 mAU/mL. Elevated PIVKA-II levels were also strongly correlated with PVTT (odds ratio [OR] 4.89, P=0.020).

MVI is an independent risk factor for early recurrence in HCC, and impacts prognosis. At a cut-off level of >40 mAU/mL, PIVKA-II was an independent predictor of MVI (hazard ratio [HR] 3.77, 95% CI 1.31–10.88, P=0.014), which in turn is a risk factor for recurrence. Two hundred and seventeen patients with small HCC ≤3 cm, who had three nodules without radiological evidence of vascular invasion, were retrospectively assessed. PIVKA-II of >100 mAU/mL (and AFP of >100 ng/mL) predicted pathological MVI. In another study, HCC with a single ≤3 cm nodule and PIVKA-II level of ≥40 mAU/mL was an independent predictor of MVI (OR 1.79, P=0.0126), as were AFP levels of ≥200 ng/mL (OR 1.82, P=0.0466), and tumours of ≥2 cm (OR 1.84, P=0.0052).

Statement 4: PIVKA-II measurements, before and after curative treatment (resection and radio-frequency ablation [RFA]), are useful for monitoring treatment outcomes and recurrence

Agreement: Strongly agree

Pre-treatment PIVKA-II levels and decrease of PIVKA-II after treatment predicted treatment outcomes for OS and recurrence-free survival (RFS). The changes in PIVKA-II levels appear to have better accuracy than AFP alone; however, combining both tumour markers resulted in better accuracy. PIVKA-II responses have also demonstrated independence in predicting recurrence in very early HCC.

In a meta-analysis that included 15 cohorts with 5,647 patients, pre-RFA elevated PIVKA-II significantly predicted poorer OS and RFS (HR 1.59; 95% CI 1.40–1.82; P<0.001), and (HR 1.76; 95% CI 1.42–2.17; P<0.001), while a significantly large reduction of PIVKA-II (and AFP) levels post RFA, was associated with a reduction of recurrence rate and improving survival time. A PIVKA-II level of ≥100 mAU/mL was an independent risk factor along with AFP ≥15 ng/mL and tumour size ≥2 cm, with relative risks (RR) of 4.19, P=0.003; 3.05, P=0.02, and 3.34, P=0.03, respectively, for recurrence post RFA.

Pre-operative elevation of both AFP and PIVKA-II was significantly associated with the development of recurrence, and shorter disease-free survival than the elevation of only one marker (cut-off levels were 20 ng/mL and 40 mAU/mL, respectively). A systematic review (n=12 studies) that included studies measuring AFP and PIVKA-II responses to various treatment modalities, including liver resection, revealed that a high pre-treatment level of both tests was associated with higher risk of recurrence, including early recurrence (within six months). Higher pre-treatment AFP and PIVKA-II levels were also associated with unfavourable tumour characteristics, MVI, and multiple tumours. PIVKA-II and AFP levels that did not decline at three months post resection, and had shorter doubling times, also predicted recurrence and significantly poorer OS.

Elevated PIVKA-II levels, before and after resection, independently predicted disease-free survival and OS, as did tumour size and number, and MVI. PIVKA-II levels of >46 mAU/mL were a risk factor for early recurrence (P=0.002), along with the magnitude of tumour necrosis (P=0.012), and the presence of MVI (P=0.029) post curative resection. More patients had elevated PIVKA-II (>40 mAU/mL) than higher AFP levels (>20 ng/mL) at recurrence, and PIVKA-II had better specificity and sensitivity than AFP (92.3% vs. 87.2%, and 74.1% vs. 40.7%, respectively). However, a variation in the difference of levels pre- and post-treatment suggests that combining PIVKA-II and AFP may improve the earlier detection of recurrence.

In patients with very early-stage HCC (BCLC Stage 0–A),...
pre-resection PIVKA-II levels of ≥373.51 mAU/mL demonstrated a strong independent factor in predicting shorter time to progression, post resection. In patients without macroscopic vascular invasion, a pre-resection PIVKA-II level of >445 mAU/mL was an independent risk factor for postoperative tumour recurrence. The tumour-free survival rates at 1 and 2 years for patients with pre-treatment PIVKA-II levels of ≥445 mAU/mL (90.4% and 70.7% respectively) were significantly higher than those with elevated pre-operative levels (73.2% and 50.5%, P=0.048).

Statement 5: PIVKA-II measurements before and after intra-arterial treatment (transarterial chemoembolization [TACE] and Yttrium-90 transarterial radioembolization [TARE]) are clinically useful to indicate response

Agreement: Strongly agree

Pre- and post-treatment PIVKA-II levels have demonstrated an association with treatment outcomes. Lower pre-treatment PIVKA-II levels appear to predict better OS, whilst a good serological response (usually taken as a reduction of ≥20–50% of pre-treatment levels) has correlated with radiological response, better OS and progression-free survival (PFS), and complete (CR) and partial (PR) responses. However, the current data may not be robust enough to support a strong recommendation for pre- and post-intra-arterial treatment PIVKA-II testing.

PIVKA-II and AFP responders to TACE had better time to progression and OS than non-responders (P<0.001). When the cut-off levels for PIVKA-II and AFP were set at ≥60 mAU/mL and ≥200 ng/mL, respectively, and serological response to ≥50.0% reduction from baseline, serological responders correlated with radiologic responders and had better OS than non-responders (HR: PIVKA-II 3.40 and AFP 4.70; all P<0.001, respectively). Differences in pre-treatment and 3- and 6-month post-treatment AFP and PIVKA-II levels independently predicted OS, with combined responders doing significantly better than either alone (P=0.011). Reductions in AFP and PIVKA-II, after TACE treatment were higher in patients with CR and PR vs. stable and progressive disease, but was not associated with better PFS.

PIVKA-II monitoring assists with predicting OS and PFS in TACE. Low pre-treatment PIVKA-II was associated with increased OS (HR 0.65, 95% CI 0.44–0.96), and its response post-TACE of ≥20.0–50.0% reduction was associated with increased OS and PFS (HR 0.39, 95% CI 0.22–0.70 and 0.42, 95% CI 0.23–0.74, respectively). Patients with elevated PIVKA-II levels pre- and post-TACE had poorer survival than those with elevated pre-TACE levels and low post-TACE levels (HR 8.47; P<0.0001). As the PIVKA-II response was significantly predictive of OS in patients with a high PIVKA-II level at baseline (HR 3.20; P<0.001), it could be a surrogate of immediate and prolonged clinical outcomes post-TACE, especially in patients with high baseline PIVKA-II levels. Monitoring PIVKA-II level trends might be helpful, as it was also strongly associated with objective response rates and disease control rates (P=0.009 and P=0.004, respectively).

A study to determine the predictive values of AFP, PIVKA-II, and modified Response Evaluation Criteria In Solid Tumours (RECIST) response post-TARE, included 63 Child-Pugh Class A patients with AFP >20 ng/mL and PIVKA-II >20 mAU/mL who were treated with TARE. Responses to AFP and PIVKA-II were defined as >50.0% decrease in levels from baseline. Response based on modified RECIST scores was defined as a complete or partial response. PIVKA-II responders had better survival at three and six months, although AFP and modified RECIST responders also demonstrated better survival at three months. The median OS between AFP and PIVKA responders and non-responders at three months were 75.8 months vs. 7.6 months for AFP and 75.8 months vs. 7.1 months for PPIVKA-II, respectively.

Statement 6: Pre-liver transplant PIVKA-II levels are associated with the risk of post-operative HCC recurrence, potentially facilitating the patient selection

Agreement: Strongly agree

As HCC recurrence after liver transplant is strongly associated with HCC histological grade, as well as AFP and PIVKA-II levels, measuring pre-liver transplant levels for both these markers can serve as an indication of the expected outcomes in patients who might need the operation. The interest in the association between pre-operative PIVKA-II levels and outcomes post-liver transplant is still fairly recent, and is based on the findings from a systematic review. The number of studies of quality focusing on this association is very small and were mainly done in the Japanese population. Therefore, more studies are required for a stronger recommendation.

Pre-operative AFP and PIVKA-II (cut-off values 300 ng/mL and 300 mAU/mL, respectively) were significantly associated with recurrence, post liver transplant, and their combination was a better predictor than either alone. Patients with far
advanced HCC and low AFP and PIVKA-II levels (≤300 for both), had significantly better 5-year OS and RFS rates (47.8% and 53.4%, respectively) than those with elevated AFP and PIVKA-II levels (21.0% and 10.8%, respectively). Hence, there might be a role for combining AFP and PIVKA-II levels in patients with advanced HCC, to facilitate selection for transplantation.

Pre-operative PIVKA-II (cut-off values 300–442 mAU/mL) impacted post-liver transplant HCC recurrence. Elevated levels were associated with shorter disease-free survival (HR 5.04, 95% CI 3.32–7.67; P<0.001) indicating that the inclusion of pre-liver transplant PIVKA-II levels could improve the eligibility of HCC patients for liver transplantation.66

Pre-liver transplant PIVKA-II levels were also inversely correlated with patient survival post liver transplant.58 When PIVKA-II was ≤100 mAU/mL (n=336), the survival at 1 year was 96.2%, at 3 years was 92.3%, and at 5 years was 91.0%; however, when the levels were >1,000 mAU/mL (n=44), the rates dropped drastically to 71.9%, 37.1%, and 29.7%, respectively. Hence, the Japanese Liver Transplantation Study Group proposed the incorporation of pre-operative AFP and PIVKA-II levels at ≤200 ng/mL and ≤100 mAU/mL, respectively, taken together to facilitate patient selection for liver transplantation.

DISCUSSION

Based on the available evidence and experts’ opinions, PIVKA-II in combination with AFP and US shows potential benefit for surveillance of small and AFP-negative HCC. However, the addition of PIVKA-II for surveillance of small HCC requires strong evidence, such as a prospective longitudinal study comparing the effectiveness of US and AFP to US, AFP and PIVKA-II. On the other hand, the evidence in utilising PIVKA-II in detecting AFP-negative HCC appears to be stronger. It is important to note the lack of evidence to suggest that the combination of AFP, PIVKA-II and US is superior to utilising AFP and US for the detection of AFP-negative HCC.

A significant limitation for reaching these consensus statements was the paucity of studies with good levels of evidence as recommended by the EDRN and ILCA. Of the four retrieved studies, only one compared PIVKA-II, AFP and their combination, while the rest had included AFP-L3 and/or the GALAD score. However, the phase 3, Level 2a study by Lok et al.13 demonstrated significant value in combining PIVKA-II and AFP for detection of early HCC.

Viral hepatitis-related HCC is a leading cause of HCC in Asia, particularly chronic hepatitis B virus infection.3 However, with the availability of a new generation of treatments, chronic hepatitis B viral replication is effectively suppressed and hepatitis C virus infections cured.69 Antiviral treatment causes viral suppression and reduces the inflammation, which consequently lowers the AFP levels. Hence, cut-off levels for AFP for detection of HCC will have to be lower than the presently accepted thresholds.60,61 However, more evidence and a consensus are needed to determine the optimal AFP cut-off values for patients treated with antivirals. The addition of PIVKA-II has demonstrated good detection rates in AFP-negative HCC and, therefore, should be incorporated where feasible.

The data also show that PIVKA-II and AFP perform differently depending on the aetiology of the HCC, and also the cut-off values used11,13,22,23,27. In patients with chronic hepatitis B-related HCC, PIVKA-II appears to do better than AFP, but on the other hand, the number of studies is comparatively small compared to the evidence for AFP. Therefore, more studies with adequate sample sizes for the different aetiologies for HCC should be performed to further strengthen the position and role of PIVKA-II+AFP in the surveillance of HCC. There is also a lack of studies involving hepatitis C-related HCC patients, as most studies have focussed on chronic hepatitis B-related HCC.

Non-alcoholic fatty liver disease (NAFLD) is a very common disease and also a risk factor for HCC.3 With the progressive efforts to reduce and potentially eliminate viral hepatitis, and the rampant increase of diabetes mellitus and obesity, NAFLD could become an important cause of HCC.3 NAFLD has been associated with a 2.6-fold increased risk of HCC, whilst diabetes alone, a 2.3-fold increased risk.1 Furthermore, NAFLD-related HCC frequently occurs without cirrhosis, making patient selection and execution of surveillance programs using AFP and US difficult.62 A few studies have demonstrated the reliability of PIVKA-II in detecting HCC in patients with NAFLD and non-alcoholic steatohepatitis (NASH).62,63 As there were only a few studies performed to determine the utility of PIVKA-II for screening high-risk NAFLD/NASH patients, the experts agreed that a consensus could not be reached at the present time, even though PIVKA-II in combination with age, gender, AFP and AFP-L3 (the GALAD score) might have a role to play in the future.63
The consensus for the utilisation of PIVKA-II for monitoring the response of curative treatments, particularly post-ressection and RFA, was much stronger. The evidence demonstrating the benefits of PIVKA-II in combination with AFP, pre- and post-local curative treatments, was consistent, leading to all of the experts being in agreement (100% agreement) that PIVKA-II can be a recommended biomarker. However, its utility in pre-liver transplant patients requires stronger evidence involving larger sample sizes and the inclusion of populations beyond Japan.

The experts anticipate challenges in implementing PIVKA-II locally. Unlike AFP, PIVKA-II lacks international standardisation, and its values are dependent on the assays used. Furthermore, the cut-off values used in clinical studies vary widely from >20 mAU/mL to >1,000 mAU/mL, making it challenging to implement across different laboratories. To ensure a measure of standardisation and optimisation of PIVKA-II utility, localisation of the reference interval and cut-off values will be required. Conducting localised, small-scale clinical validation studies will help establish the performance and assay-specific cut-off values of PIVKA-II in the local population. Additionally, it will be important for clinicians to understand that baseline PIVKA-II value may be inadequate to detect early HCC and serial monitoring of the biomarker level should be done.

At present, only a few countries like Japan and Taiwan have PIVKA-II reimbursement programs. Hence, the cost and subsequent funding of PIVKA-II will be a major challenge for adopting it in HCC surveillance. To date, cost-effectiveness studies for combining PIVKA-II and AFP for HCC surveillance and monitoring are lacking. Hence, to improve the adoption of PIVKA-II testing as part of HCC surveillance, health economic studies at regional or national levels are required, in order to justify its use. Another area that requires more study is the timing of PIVKA-II elevation and its correlation to HCC development. Longitudinal studies are key to determining this, as the data could influence the schedule for screening, and hence the number of expected tests and its overall cost.

Improving awareness of PIVKA-II among relevant healthcare providers will be essential for its proper use and the interpretation of its results together with AFP levels, tumour clinical characteristics, and factors that might affect its value. Medical education programs, health economic studies, and studies localising PIVKA-II values, will have significant roles before driving its endorsement into regional and local guidelines.

The experts also discussed the utility of using PIVKA-II levels to guide recall for confirmation of HCC and implementation of PIVKA-II in laboratories. Generally, the presence of one or two parameters (elevated AFP levels, elevated PIVKA-II levels, and US findings) could guide recall. Recall of patients might be warranted if elevated AFP and PIVKA-II levels raise suspicion of very small tumours. However, this will depend on the magnitude of the elevation of the biomarkers’ levels. In the absence of US findings, other causes of elevated PIVKA-II and AFP levels should be ruled out, followed by serial monitoring of their levels.

The consensus reached by the experts is strengthened by their collectively vast experience in managing patients with HCC and input from experts who are heads of national laboratories. Articles were also extensively collated for their review, and included meta-analyses and randomised controlled studies, which provide a high level of evidence. On the other hand, the experts agreed that more data, including evidence from cost-effectiveness and longitudinal studies, as well as clinical experience, could advance a stronger recommendation for PIVKA-II utilisation in the region. Currently, other than Japan and South Korea, most countries in the Asia-Pacific region have moderate-to-minimal experience with utilising PIVKA-II extensively in practice.

CONCLUSION

PIVKA-II in combination with AFP and US will be clinically useful in the Asia-Pacific region in surveillance, especially for those with small and AFP-negative HCC, and more so in predicting treatment outcomes in HCC patients. More evidence is required, and stronger consensus at an international level remains to standardise cut-off values and tighten its reference range, in order to support easier applicability of the test. There is also a need for cost-effectiveness studies to justify its use on a broad scale.

Authors’ contribution

All authors contributed to the discussion and consensus process. The first draft of the manuscript was written by Kim Do-Young and Chan Henry Lik Yuen. All authors commented on the previous versions of the manuscript, and read and approved the final version.
Acknowledgements

The writing committee wishes to acknowledge Roche Diagnostics for supporting the manuscript writing via an educational grant.

Conflicts of Interest

This study did not include the use of human or animal subjects.

Below is the list of conflict-of-interest statements: Kim Do-Young, Toan Bao Nguyen, Setiawan Lyana, Huyen Nguyen Nguyen, Mohamed Rosmawati, Hai Thi Thanh Nguyen and Lee Woo-Chang declare that they have no conflict of interest.

Tan Chee-Kiat has received honoraria made to his institution for lectures, presentations, speakers bureaus, manuscript writing and education events from Abbott Laboratories, Astellas and Gilead Sciences. He has also received payment made to his institution for participation on a Data Safety Monitoring Board and Advisory Board for Abbott Laboratories, Bayer, Eisai, Gilead Sciences and Roche Diagnostics.

Hasan Irsan has received honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Eisai and Roche.

Yu Ming-Lung has received grants from Abbott, BMS, Gilead and Merck. He has also received consulting fees and honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Abbott, Abbvie, BMS, Gilead, IPSEN, Merck, Roche and Roche Diagnostics.

Namiki Izumi has received honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Chugai, Eisai and Takeda.

Chow Pierce Kah-Hoe has received grants from AMiLi, MiRXES, Perspectum and Roche. He has received consulting fees for Beigene, Omega Therapeutics and Worrell (LLC), and honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Abbott, AstraZeneca, Eisai, Roche and Sirtex Medical. He has a patent submitted for “A system and method for classify cancer patients into appropriate cancer treatment groups and compounds for treating the patients. Pub. No.: WO/2019/108135 A1 International; Application No.: PCT/SG2018/050585. He has received payment for participating on Data Safety monitoring Boards and Advisory Boards from AstraZeneca, AUM-Bioscience, Genentech, Roche, Singapore-Samsung Medical Centre (SG-SMC) Joint Lab and Sirtex Medical. In addition, he declares having stock or stock options in AVATAMED Pte. Ltd.

Chan Stephen Lam has received consulting fees and honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from AstraZeneca, Eisai and MSD. He has participated on a Data Safety Monitoring Board and Advisory Board for AstraZeneca.

Tanwandee Tawesak has received grants from Gilead, Janssen, MSD, Roche and Vir Biotech.

Lee Teng-Yu has received grants from Gilead, MSD and Roche. He has received consulting fees and honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from AbbVie, BMS, Eisai, Gilead and Roche. He has participated on Advisory Boards for BMS, Eisai, Gilead and Roche.

Yang Tian has received honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Abbott and Roche.

Chan Henry Lik Yuen has received consulting fees from AbbVie, Aligos, Arbutus, Hepion, Gilead, GSK, Janssen, Roche, Vaccitech, Vir Biotechnology and Virion Therapeutics. He has received honoraria for lectures, presentations, speakers’ bureaus, manuscript writing and education events from Gilead, Roche and Viatris, and received support for attending an overseas conference from Gilead. He has participated on Data Safety Monitoring Boards for Aligos, Roche and Vaccitech.

REFERENCES


Neuropilins as potential biomarkers in hepatocellular carcinoma: a systematic review of basic and clinical implications

Paula Fernández-Palanca¹,², Tania Payo-Serafin¹,², Carolina Méndez-Blanco¹,², Beatriz San-Miguel¹,², María J. Tuñón¹,², Javier González-Gallego¹,², and José L. Mauriz¹,²

¹Institute of Biomedicine (IBIOMED), Universidad de León, León; ²Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

Hepatocellular carcinoma (HCC) is one of the most common and deadly cancers worldwide and is characterized by complex molecular carcinogenesis. Neuropilins (NRPs) NRP1 and NRP2 are the receptors of multiple proteins involved in key signaling pathways associated with tumor progression. We aimed to systematically review all the available findings on their role in HCC. We searched the Scopus, Web of Science (WOS), PubMed, Cochrane and Embase databases for articles evaluating NRPs in preclinical or clinical HCC models. This study was registered in PROSPERO (CRD42022349774) and include 49 studies. Multiple cellular and molecular processes have been associated with one or both NRPs, indicating that they are potential diagnostic and prognostic biomarkers in HCC patients. Mainly NRP1 has been shown to promote tumor cell survival and progression by modulating several signaling pathways. NRPs mainly regulate angiogenesis, invasion and migration and have shown to induce invasion and metastasis. They also regulate the immune response and tumor microenvironment, showing a crucial interplay with the hypoxia response and microRNAs in HCC. Altogether, NRP1 and NRP2 are potential biomarkers and therapeutic targets, providing novel insight into the clinical landscape of HCC patients. (Clin Mol Hepatol 2023;29:293-319)

Keywords: Biomarker; Hepatocellular carcinoma; Neuropilins; Systematic review

INTRODUCTION

Liver tumors are common and deadly cancer, with increasing incidence and mortality rates.¹ Hepatocellular carcinoma (HCC) is the main primary liver tumor type, accounting for approximately 90% of all cases.² HCC is a heterogeneous cancer mainly diagnosed at advanced stages. Its survival rate remains very low despite the available systematic therapies that only increase the survival probability of patients 1–2 years.²,³ It is characterized by unique molecular carcinogenesis involving multiple modulators, signaling pathways, and mechanisms.²,⁴ All of these factors contribute to the difficulty in understanding HCC development and progression. Therefore, further studies are required to provide clearer insights into its molecular mediators to develop effective targeted therapies and improve the outcomes of HCC patients.²,⁴ The molecular pathogenesis of HCC is generally complex,

Corresponding author : José L. Mauriz
Institute of Biomedicine (IBIOMED), Universidad de León, Campus de Vegazana s/n, 24071, León, Spain
Tel: +34 987291981, Fax: +34 987291276, E-mail: jl.mauriz@unileon.es
https://orcid.org/0000-0003-3160-8599

Editor: Bo Hyun Kim, National Cancer Center, Korea
Received: Nov. 28, 2022 / Revised: Jan. 16, 2023 / Accepted: Jan. 31, 2023

Copyright © 2023 by Korean Association for the Study of the Liver
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
with increasing numbers of molecules reported to participate in the development, progression, and drug sensitivity of HCC cells. Neuropilins (NRPs) have recently been the focus of several studies due to their involvement in numerous cellular processes in cancer, such as cell proliferation, migration, invasion, and angiogenesis. NRPs are 130–140 kDa type-1 membrane glycoproteins with extracellular, transmembrane, and cytoplasmic domains. They act as co-receptors for proteins such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and transforming growth factor β (TGF-β). There are two NRPs (NRP1 and NRP2) encoded by different genes on independent chromosomes but with a common domain structure. While both NRPs share some characteristics and ligands, they differ in their tissue distribution and modulated signaling pathways. Moreover, while NRP1 has been widely studied and characterized, fewer studies have focused on NRP2. Nevertheless, the role played by both NRPs in cancer, including HCC, has recently become of interest due to their strong correlation with key cellular and molecular mechanisms involved in tumor progression.

Interestingly, despite increasing studies evaluating the role of NRP1 and NRP2 in human HCC, no attempt has been made to compile the main findings of their potential roles in the HCC tumor landscape. Therefore, we aimed to summarize through a systematic literature review the findings of published studies using preclinical and/or clinical HCC models and evaluating one or both NRPs.

**MATERIALS AND METHODS**

**Protocol and registration**

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Supplementary Tables 1 and 2) and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42022349774).

**Literature search strategy**

A comprehensive literature search was performed in the Web of Science, Scopus, PubMed, Embase and Cochrane Library databases of articles published up to August 31, 2022, identifying 293 articles (Fig. 1). This search strategy combined the search terms “NRP”, “NRP1”, “NRP2”, and “HCC”, in the search queries used for each database (Supplementary Table 3).

**Inclusion and exclusion criteria**

Studies that met the following eligibility criteria were included in this systematic review: (1) they involved human patients diagnosed with HCC, animal HCC models, or in vitro primary or genetically-modified HCC cell line models; (2) they determined NRPs expression or derived effects from modifying NRPs; (3) they evaluated tumor-associated processes or characteristics. Studies that met the following exclusion criteria were removed during the study selection process: (1) they involved human patients without an HCC diagnosis, or unsuitable or poorly-described HCC models; (2) they were reviews or similar articles; (3) they did not evaluate or report NRPs expression or derived effects from modifying NRPs; (4) their full-text was not available in English.

**Study selection**

Two authors independently performed the study selection process, and discrepancies were resolved by a third author based on a consensus.

Duplicates among the original articles identified in the initial search of all databases were removed. Next, the articles were subjected to an initial screening by title and abstract based on exclusion criteria. Then, the remaining articles' full
texts were screened against the eligibility criteria, and those meeting the inclusion criteria were identified and included in the qualitative analysis.

**Data collection and analysis**

Three authors independently extracted data from the included studies, compiling the information in separate tables for preclinical and human studies.

The following information was included from preclinical studies: the first author’s name, publication year, model used, sample type, NRP subtype, measurement method, NRP alteration, associated cellular process, and alterations observed.

The following information was included from human studies: the first author’s name, publication year, case and control number, related etiology, mean age, sample type, NRP sub-
type, determination sample type, tumor tissue expression, and clinical involvement.

RESULTS

Study selection and characteristics

The complete literature search and study selection process was conducted as described in Figure 1. The comprehensive search identified 293 unique articles after removing 118 duplicates between the five databases. Both inclusion and exclusion criteria were used to select studies that fit this study’s scope, identifying 49 eligible articles for inclusion in the systematic review.

The number of published articles assessing the NRPs’ role in different HCC models has increased in recent years, with most studies conducted since 2010 (Supplementary Fig. 1A). Curiously, in vitro studies have been replaced by in vivo studies using animal models or human samples. Moreover, when separately considering published studies on HCC patients (Supplementary Fig. 1B) and animals (Supplementary Fig. 1C), we observed an appreciable increase in published studies involving human patients in the last three years, along with increases in the number of patients used (Supplementary Fig. 1B). However, while in vivo models have been constantly used over time, their sample sizes have increased in recent years (Supplementary Fig. 1C). The main characteristics of all included articles are summarized separately for studies involving preclinical models (Table 1A) and human HCC samples (Table 1B).

All 49 of the included studies evaluated the role of NRP1 and/or NRP2 in HCC, of which 11 used only in vitro models (22.45%), four only animal models (8.16%), seven both in vitro and in vivo models (14.29%), 19 only human HCC patient samples (38.78%) and eight preclinical and clinical samples (16.33%). There was a notable difference in the number of studies examining each NRP subtype. Only four of the 49 included studies examined NRP2 (8.16%), compared to 40 that examined NRP1 (81.63%), five examined both NRPs (10.20%). Most (22/27 [81.48%]) clinical articles on human patients evaluated NRP protein expression directly in tumor tissue. While detecting potential tumor markers in serum samples is an important tool for improving HCC diagnosis, only five studies analyzed serum NRP1 levels in human patients.

NRP measurements in preclinical models used different approaches. The most common was the real-time reverse transcription polymerase chain reaction (qRT-PCR; 33.33%), followed by western blot (16.67%), qRT-PCR and Western blot (20.00%), immunofluorescence microscopy (6.67%), and all three techniques (10.00%). In addition, some individual studies used different methodologies including qRT-PCR and immunofluorescence microscopy (3.33%), western blot and immunofluorescence microscopy (3.33%), and mass spectrometry (3.33%). One of the 30 preclinical studies did not assess NRP expression (3.33%).

In addition, most included studies targeted one NRP to assess the derived effects. However, their targeting strategies varied widely, from genetic silencing to using inhibitors or antibodies (Table 2). Given the many articles evaluating the NRPs’ role in various cellular and molecular processes in HCC, their main findings have been organized and described in the following sections.

NRPAs diagnostic tissue or serum biomarkers

While NRPs were initially identified in the nervous system as axon guidance regulators, recent studies have reported multiple molecular functions. Late diagnosis is one of the leading causes of HCC patients’ high mortality rate due to the absence of highly sensitive and specific biomarkers. Therefore, an increasing number of studies have focused on the potential use of NRPs as tissue or serum biomarkers evaluating their expression levels in tumor and healthy liver samples from HCC patients to improve the current diagnostic tools for HCC diagnosis.

Studies have determined NRP protein levels in tumor tissue from HCC patients, finding NRP1 to be overexpressed compared to healthy liver tissue and in high-risk HCC patients. In contrast, Kitagawa et al. did not find a difference, while lower NRP1 levels in tumor compared to peritumoral tissue have also been reported. Similarly, NRP1 levels were increased in three HCC cell lines compared to the normal liver L02 and Hep3B and HepG2. Contrariwise, NRP2 expression in HCC tissue has been analyzed to a lesser extent. Lower NRP2 expression was found in the HCC nodules versus the healthy liver tissue. However, contradictory results have been reported for NRP2. While its urinary levels were decreased in a rat HCC model, an in vitro study found it was overexpressed in three human HCC cell lines. These find-
Table 1. Main characteristics of the included studies employing (A) preclinical and (B) clinical models

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Preclinical studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liao et al.</td>
<td>2008</td>
<td>In vitro</td>
<td>Mahlavu, Huh-7, SK-Hep1 and HEK293T cell lines</td>
<td>NRP1</td>
<td>RT-PCR</td>
<td></td>
<td>NRP1 silencing</td>
<td>Cell migration</td>
<td>↓ Cell migration</td>
</tr>
<tr>
<td>Bergé et al.</td>
<td>2010</td>
<td>In vitro</td>
<td>HepG2, SK-HEP-1 and PLC/PRF/5 cell lines</td>
<td>NRP1</td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Angiogenesis</td>
<td>No alterations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vivo Transgenic HCC C57BL/6 mice</td>
<td>NRP1</td>
<td>ICC</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Tumor progression/development</td>
<td>↓ Tumor growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Angiogenesis</td>
<td>Inhibition of tumor vasculature remodeling</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Immune-related response</td>
<td>↑ IFN-γ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>No changes in IFN-β and IL-12</td>
<td></td>
</tr>
<tr>
<td>Lee et al.</td>
<td>2010</td>
<td>In vitro</td>
<td>HepG2 cell line</td>
<td>NRP1</td>
<td>qRT-PCR</td>
<td></td>
<td>TCDD exposure PRMT1 and PRMT4 co-inhibition by silencing</td>
<td>Xenobiotic toxicity associated to cancer</td>
<td>↑ NRP1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Cell proliferation</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Apoptosis</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Cell migration</td>
<td>↓ Cell migration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Tube formation ability</td>
<td>↓ Tumor growth by both siR NRP1 and siR Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Cell proliferation</td>
<td>↓ Cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Apoptosis</td>
<td>↓ Apoptosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Angiogenesis</td>
<td>No changes in pro-angiogenic factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Immune-related response</td>
<td>↑ TNF-α</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td></td>
<td>↑ IFN-β</td>
</tr>
</tbody>
</table>
### Table 1. Continued

#### A. Preclinical studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model Description</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergé et al.</td>
<td>2011</td>
<td>In vitro HepG2, SK-HEP-1 and PLC/PRF/5 cell lines</td>
<td>Western blot</td>
<td>NRP1</td>
<td>-</td>
<td>Peptide N-derived inhibition</td>
<td>Cell proliferation, Apoptosis, Angiogenesis, Cell viability, Cleaved caspase-3, Capillary-like structure formation, Total tube length, Tubular network area, Liver volume and weight, Nodule size and Ki67 staining, TUNEL staining, Inhibition of tumor vasculature remodeling, Microvessels number, Total microvessels length, Mean blood flow velocity of hepatic and mesenteric arteries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Devbhandari et al.</td>
<td>2011</td>
<td>In vitro HCCLM3 cell line</td>
<td>Western blot and mass spectrometry</td>
<td>NRP1</td>
<td>-</td>
<td>Peptide N-derived inhibition</td>
<td>Tumor progression, Development, Apoptosis, TUNEL staining, Tumor vasculature, Inhibition of tumor vasculature remodeling, Microvessels number, Total microvessels length, Mean blood flow velocity of hepatic and mesenteric arteries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee et al.</td>
<td>2011</td>
<td>In vitro HepG2 and Huh-7 cell lines</td>
<td>Western blot</td>
<td>NRP1</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Cell death, No alterations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raskopf et al.</td>
<td>2012</td>
<td>In vitro Mouse Hepa129 cell line</td>
<td>qRT-PCR</td>
<td>NRP1</td>
<td>-</td>
<td>Downregulation derived from VEGF silencing</td>
<td>Angiogenesis, VEGF silencing decreased NRP1 expression, VEGF silencing decreased NRP2 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>qRT-PCR</td>
<td>NRP2</td>
<td>-</td>
<td>Downregulation derived from VEGF silencing</td>
<td>Angiogenesis, VEGF silencing decreased NRP2 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yaqoob et al.</td>
<td>2012</td>
<td>In vitro HepG2 cell line</td>
<td>Western blot</td>
<td>NRP1</td>
<td>-</td>
<td>HSC overexpressing NRP1</td>
<td>Cell proliferation, Conditioned matrix from HSC increased Ki67 staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chishti et al.</td>
<td>2013</td>
<td>In vivo Sprague Dawley rats with drug-induced HCC</td>
<td>qRT-PCR</td>
<td>NRP1</td>
<td>Upregulated in HCC animals</td>
<td>NRP expression</td>
<td>1 NRP1 in HCC, NRP1 in HCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The table continues on the next page.
### Table 1. Preclinical studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu and Xia</td>
<td>2013</td>
<td>\textit{In vitro}</td>
<td>HCCLM6 cell line</td>
<td>NRP1</td>
<td>Western blot</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Cell proliferation</td>
<td>↓ Cell growth rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{In vivo}</td>
<td>HCCLM6 xenograft nude mice</td>
<td>NRP1</td>
<td>qRT-PCR, Western blot and ICC</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Tumor progression/development</td>
<td>↓ Tumor size</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor weight</td>
<td>↓ Tumor weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor vasculature/Invasion</td>
<td>↓ Neovascularization</td>
</tr>
<tr>
<td>Horwitz et al.</td>
<td>2014</td>
<td>\textit{In vivo}</td>
<td>Mdr2-/- mice</td>
<td>NRP1 and NRP2</td>
<td>qPCR</td>
<td>-</td>
<td>NR</td>
<td>Immune-related response</td>
<td>↑ NRP1 and NRP2 in macrophages</td>
</tr>
<tr>
<td>Zhuang et al.</td>
<td>2014</td>
<td>\textit{In vitro}</td>
<td>HCCLM3 HCC cell line</td>
<td>NRP1</td>
<td>ICC and qRT-PCR</td>
<td>-</td>
<td>CoCl₂ treatment</td>
<td>Hypoxia response</td>
<td>↓ NRP1 in L02 cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{In vivo}</td>
<td>and L02 healthy liver cell line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ NRP1 time-dependent in peritumoral tissue while ↓ Hypoxia</td>
</tr>
<tr>
<td>Liu et al.</td>
<td>2015</td>
<td>\textit{In vitro}</td>
<td>PLC/PRF/5 and HuH-7 cell lines</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>-</td>
<td>Overexpression derived from Inh-148b</td>
<td>Angiogenesis</td>
<td>↑ Tube formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Downregulation derived from miR-148b</td>
<td>Angiogenesis</td>
<td>↓ Tube formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MicroRNA modulation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CSC properties</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 is a target of miR-148b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 expression in side population cells of HCC cell lines</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cell division, tumor weight, tumor volume</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cell division, tumor weight, tumor volume</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MicroRNA modulation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 is a target of miR-148b</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1. Continued**
### A. Preclinical studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wittmann et al.</td>
<td>2015</td>
<td>In vitro</td>
<td>3sp, SNU-398, SNU-423, SNU-449, SNU-475, FLC-4 cell lines</td>
<td>NRP2</td>
<td>qRT-PCR and Western blot</td>
<td>-</td>
<td>-</td>
<td>Mesenchymal phenotype</td>
<td>NRP2 was correlated with mesenchymal phenotype</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP2 silencing</td>
<td>Migration and invasion abilities</td>
<td>Migration and invasion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGF-β signaling</td>
<td>NRP2 correlated with TGF-β</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TGF-β treatment</td>
<td>NRP2</td>
<td></td>
</tr>
<tr>
<td>Kisseleva et al.</td>
<td>2016</td>
<td>In vivo</td>
<td>Hepatoma 22a C3HA mice</td>
<td>NRP1</td>
<td>qRT-PCR and flow cytometry</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 NRP1 in thymocytes</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>2016</td>
<td>In vitro</td>
<td>Hep3B and HepG2 cell lines</td>
<td>NRP1</td>
<td>qRT-PCR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 NRP1</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2016</td>
<td>In vitro</td>
<td>Bel-7402, SMMC-7721 and HepG2 cell lines, and L02 healthy liver cell line</td>
<td>NRP1</td>
<td>qRT-PCR, Western blot and ICC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 NRR1 in HCC cell lines</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Metastasis</td>
<td>1 NRR1 in high-metastatic cell lines</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2017</td>
<td>In vitro</td>
<td>HepG2 cell line</td>
<td>NRP1</td>
<td>GO functional enrichment analysis</td>
<td>-</td>
<td>miRNA-124 transfection</td>
<td>Axon guidance pathway</td>
<td>Enrichment of NRP1</td>
</tr>
</tbody>
</table>
### A. Preclinical studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al. 62</td>
<td>2017</td>
<td>In vitro</td>
<td>HepG2 and LX2 coculture</td>
<td>NRP1</td>
<td>Western blot and ICC</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Cell proliferation</td>
<td>↓ Cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Migration and invasion</td>
<td>↓ Cell migration and invasion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor progression/development</td>
<td>↓ Tumor volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ α-SMA staining</td>
</tr>
<tr>
<td>Lin et al. 77</td>
<td>2018</td>
<td>In vitro</td>
<td>Bel-7402 and SMMC-7721 cell lines</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>-</td>
<td>NRP1 targeting</td>
<td>Cell viability</td>
<td>↓ Cell viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRP1 silencing</td>
<td>Cell viability and colony formation</td>
<td>↑ Caspase-3/7 activity</td>
</tr>
<tr>
<td>Cheng et al. 11</td>
<td>2019</td>
<td>In vitro</td>
<td>Huh-7 cell line</td>
<td>NRP1</td>
<td>qRT-PCR</td>
<td>-</td>
<td>miR-148b overexpression</td>
<td>Migration and invasion</td>
<td>↓ Cell migration</td>
</tr>
<tr>
<td>Lv et al. 40</td>
<td>2019</td>
<td>In vitro</td>
<td>HepG2 cell line</td>
<td>NRP1</td>
<td>Western blot</td>
<td>-</td>
<td>NRP1 silencing + SSd</td>
<td>Cell viability</td>
<td>↓ Cell viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Migration and invasion</td>
<td>↓ Cell migration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NRPS is a target of SSd</td>
<td></td>
</tr>
<tr>
<td>Xu et al. 66</td>
<td>2019</td>
<td>In vitro</td>
<td>HepG2 cell line</td>
<td>NRP1</td>
<td>Confocal microscopy and flow cytometry</td>
<td>-</td>
<td>-</td>
<td>Targeted therapy</td>
<td>SUCCESSFUL DETECTION OF NRP1 ANTIBODY IN THE HCC CELL SURFACE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Localizadon of the tTF-ant-NRP1 in the tumor after 2 h of intravenous administration</td>
<td>↓ Tumor growth and progression</td>
</tr>
<tr>
<td>Arab et al. 95</td>
<td>2020</td>
<td>In vitro</td>
<td>HepG2Cyp2E1+ cell line</td>
<td>NRP1</td>
<td>-</td>
<td>-</td>
<td>Supernatant from HSC with knockdown of NRP1</td>
<td>Cell proliferation</td>
<td>↓ Lipid droplet formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ IGFBP3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ SerpinA12</td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Model</th>
<th>Sample type</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>NRP expression</th>
<th>NRP alteration</th>
<th>Cellular process associated</th>
<th>Specific alterations observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang</td>
<td>2020</td>
<td>In vitro</td>
<td>Hep3B cell line</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>-</td>
<td>Circ-ABCB10 overexpression</td>
<td>NRPI</td>
<td>↑ NRP1 expression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vivo</td>
<td>BALB/c athymic nude mice injected with Hep3B</td>
<td>NRP1</td>
<td>Western blot</td>
<td>NR</td>
<td>miR-340-5p/miR-452-5p overexpression</td>
<td>NRPI</td>
<td>↓ NRP1 expression</td>
</tr>
<tr>
<td>Ye et al.</td>
<td>2020</td>
<td>In vitro</td>
<td>HepG2, SK-Hep and Bel-7404 cell lines, and L02 healthy liver cell line</td>
<td>NRP2</td>
<td>qRT-PCR</td>
<td>NRP2 overexpression</td>
<td>-</td>
<td>-</td>
<td>↑ NRP2 expression in HCC lines</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2021</td>
<td>In vitro</td>
<td>HCCLM3 and Huh-7 cell lines</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>Stem cell properties</td>
<td>↓ Liver CSC population</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vivo</td>
<td>HepG2, HCCLM3 and Huh-7 cell lines, and L02 healthy liver cell line</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>NRP1 overexpression</td>
<td>NRP1 silencing</td>
<td>Cell proliferation</td>
<td>↓ Colony formation ability and sphere diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCCLM3 xenograft nude mice</td>
<td>NRP1</td>
<td>-</td>
<td>-</td>
<td>NRP1 silencing</td>
<td>EMT pathway</td>
<td>↓ N-cadherin and vimentin</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2021</td>
<td>In vivo</td>
<td>Wistar rats injected with rat hepatoma cell line CBRH-7919 or hepatoma cell line RH-35</td>
<td>NRP2</td>
<td>LC-MS/MS</td>
<td>NR</td>
<td>-</td>
<td>NRP2</td>
<td>↓ NRP2 in urinary samples</td>
</tr>
<tr>
<td>Li and Bao</td>
<td>2022</td>
<td>In vivo</td>
<td>H22 tumor-bearing mouse model</td>
<td>NRP1</td>
<td>Western blot</td>
<td>NR</td>
<td>IPE high dose treatment (TG group)</td>
<td>NRPI</td>
<td>↓ NRP1 expression in TG group</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number (Case/Controls)</th>
<th>Etiology related</th>
<th>Mean age</th>
<th>Sample type</th>
<th>NRP</th>
<th>Type of determination sample</th>
<th>NRP expression in tumor sample</th>
<th>Clinical involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckebaum et al.</td>
<td>2004</td>
<td>65/70</td>
<td>54 Cirrhosis, from which: 21 HCV, 10 HBV, 9 Alcohol, 13 Cryptogenic, 1 Autoimmune and 3 no cirrhosis (2 HCV and 1 HBV)</td>
<td>60±12.27 (Healthy: 57±21.56)</td>
<td>Freshly isolated peripheral blood mononuclear cells</td>
<td>NRP1</td>
<td>Serum biomarker</td>
<td>Downregulated</td>
<td>Inversely correlated with IL-10</td>
</tr>
<tr>
<td>Bergé et al.</td>
<td>2011</td>
<td>308/31</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
</tr>
<tr>
<td>Yaqoo et al.</td>
<td>2012</td>
<td>139/139</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP1 overexpression correlated with shorter OS</td>
</tr>
<tr>
<td>Kitagawa et al.</td>
<td>2013</td>
<td>12</td>
<td>6 cirrhosis, 5 chronic hepatitis, 1 normal - 3 HBV, 7 HCV, 1 both, 1 negative</td>
<td>51–81</td>
<td>Liver tissue</td>
<td>NRP1 and NRP2</td>
<td>Tissue</td>
<td>NRP1: No changes NRP2: downregulated</td>
<td>Expression in HCC tissue</td>
</tr>
<tr>
<td>Chishti et al.</td>
<td>2013</td>
<td>126/7</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
</tr>
<tr>
<td>Zhuang et al.</td>
<td>2014</td>
<td>214</td>
<td>168 cirrhosis 176 HbsAg+</td>
<td>50.45±12.4</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated in peritumoral</td>
<td>High peritumoral NRP1: lower TTR and OS</td>
</tr>
<tr>
<td>Villa et al.</td>
<td>2015</td>
<td>132</td>
<td>132 Cirrhosis 74 HCV 16 HBV 18 Alcohol 20 Dysmetabolic</td>
<td>68.25 (32–88)</td>
<td>Liver tissue</td>
<td>NRP</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP is part of a hepatic signature that constitutes an independent factor for rapid tumor growth and mortality</td>
</tr>
<tr>
<td>Wittman et al.</td>
<td>2015</td>
<td>133</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP2</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP2 overexpression correlated with higher tumor grading</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2016</td>
<td>16/16† and 105/105†</td>
<td>84 HBV</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>NRP1 overexpression correlated with intrahepatic metastasis, Edmondson grade, TNM, portal vein invasion, shorter OS and RFS</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number (Case/Controls)</th>
<th>Etiology related</th>
<th>Mean age</th>
<th>Sample type</th>
<th>NRP</th>
<th>Type of determination sample</th>
<th>NRP expression in tumor sample</th>
<th>Clinical involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong et al.10</td>
<td>2017</td>
<td>190/190</td>
<td>154 Cirrhosis 152 HBV</td>
<td>23-89</td>
<td>Liver tissue</td>
<td>NRP2</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP2 overexpression correlated with higher histological grade, absence of cirrhosis, shorter OS and DFS</td>
</tr>
<tr>
<td>Lin et al.17</td>
<td>2018</td>
<td>40/30</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC Correlated with serum AFP, γ-GT, Alb, bile acid, ALT, AST, ALP and pre-Alb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>104/80</td>
<td>NR</td>
<td>55.37±8.63</td>
<td>Serum sample</td>
<td>NRP1</td>
<td>Serum biomarker</td>
<td>Upregulated</td>
<td></td>
</tr>
<tr>
<td>Morin et al.18</td>
<td>2018</td>
<td>11</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>Marked NRP1 staining</td>
</tr>
<tr>
<td>Lyu et al.19</td>
<td>2019</td>
<td>371/50</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
</tr>
<tr>
<td>Ono et al.21</td>
<td>2020</td>
<td>41</td>
<td>7 HBV 9 HCV 9 HCV post SVR 6 Alcohol</td>
<td>72 (46-84)</td>
<td>Serum sample</td>
<td>NRP1</td>
<td>Serum biomarker (9 circulating cytokines and angiogenic factors signature)</td>
<td>NR</td>
<td>9 circulating cytokines and angiogenic factors signature associated to lower PFS, OS and early PD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9 circulating cytokines and angiogenic factors signature positively correlated with AST and ALT, and negatively with Alb</td>
</tr>
<tr>
<td>Liu et al.67</td>
<td>2020</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>TFAP4 was correlated with NRP1 as an immune marker in dendritic cells</td>
</tr>
<tr>
<td>Abdel Ghafar et al.25</td>
<td>2021</td>
<td>50/50</td>
<td>NR</td>
<td>59.2±6.7 / 57.5±7.1</td>
<td>Serum sample</td>
<td>NRP1</td>
<td>Serum biomarker</td>
<td>Upregulated</td>
<td>Correlated with OS, BCLC stages B and C, tumor number (&gt;3), tumor size (≥5 cm), vascular invasion and distant metastasis</td>
</tr>
</tbody>
</table>

Table 1.

B. Clinical studies

- **Study**: Name of the study
- **Year**: Year of publication
- **Number (Case/Controls)**: Number of cases and controls
- **Etiology related**: Etiology related to the study
- **Mean age**: Mean age of the study population
- **Sample type**: Type of sample used in the study
- **NRP**: NRP involved in the study
- **Type of determination sample**: Type of sample for determination
- **NRP expression in tumor sample**: Expression of NRP in tumor sample
- **Clinical involvement**: Clinical involvement related to NRP expression

Notes:
- NRP overexpression is associated with various clinical outcomes, including histological grade, absence of cirrhosis, shorter OS and DFS.
- Overexpression in HCC is correlated with serum AFP, γ-GT, Alb, bile acid, ALT, AST, ALP and pre-Alb.
- TFAP4 was correlated with NRP1 as an immune marker in dendritic cells.
### Table 1. Continued

**B. Clinical studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number (Case/Controls)</th>
<th>Etiology related</th>
<th>Mean age</th>
<th>Sample type</th>
<th>NRP</th>
<th>Type of determination sample</th>
<th>NRP expression in tumor sample</th>
<th>Clinical involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. 26</td>
<td>2021</td>
<td>81 (cohort 1)</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 (cohort 2)</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>239 (cohort 3)</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>NR overexpression correlated with shorter OS and vascular invasion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upexpression in patients with recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>NR overexpression in patients with recurrence</td>
<td></td>
</tr>
<tr>
<td>Li et al. 26</td>
<td>2022</td>
<td>374/50</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>High NRP1 expression in the high-risk group of HCC patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>NR overexpression with Rad51, a valuable prognosis marker in HCC</td>
<td></td>
</tr>
<tr>
<td>Liu et al. 47</td>
<td>2022</td>
<td>5/5</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1 and NRP2</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP1/NRP2-VEGFA interaction is involved in HCC tumorigenesis</td>
<td></td>
</tr>
<tr>
<td>Li et al. 25</td>
<td>2022</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Overexpression in HCC</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number (Case/Controls)</th>
<th>Etiology related</th>
<th>Mean age</th>
<th>Sample type</th>
<th>NRP</th>
<th>Type of determination sample</th>
<th>NRP expression in tumor sample</th>
<th>Clinical involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng et al.</td>
<td>2022</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>NR</td>
<td>NRP1 (as immune-related gene) was not correlated with KLRB1</td>
</tr>
<tr>
<td>Fernández-Palanca et al.</td>
<td>2022</td>
<td>1,156</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Negatively correlated with OS and RFS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-49</td>
<td>NR</td>
<td>NR</td>
<td>Serum sample</td>
<td>NRP1</td>
<td>Serum biomarker</td>
<td>Upregulated</td>
<td>Directly associated to higher venous invasion and metastasis</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2022</td>
<td>247/241</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>Upregulated</td>
<td>Correlated with higher recurrence</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2022</td>
<td>156</td>
<td>NR</td>
<td>NR</td>
<td>Liver tissue</td>
<td>NRP1</td>
<td>Tissue</td>
<td>No altered</td>
<td>Correlated with higher recurrence</td>
</tr>
</tbody>
</table>

*α-SMA, α-smooth muscle actin; AFP, alpha fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CAF, cancer-associated fibroblast; CSC, cancer stem cell; DFS, disease-free survival; γ-GT, gamma-glutamyl transpeptidase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSC, hepatic stellate cell; ICC, immunocytochemistry; IFN, interferon; IGFBP3, insulin-like growth factor binding protein-3; IHC, immunohistochemistry; IL, interleukin; IPE, Inonotus hispidus petroleum ether extract; KLRB1, killer cell lectin-like receptor B1; LC-MS/MS, liquid chromatography-tandem mass spectrometry; miRNA, microRNA; NR, not reported; NRP, neuropilin; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PRMT1, protein arginine methyltransferase 1; qRT-PCR, real-time reverse transcription polymerase chain reaction; RFS, recurrence-free survival; SSd, Saikosaponin d; SVR, sustained virologic response; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TEAD, TEA domain transcription factor; TEC, tumor-associated endothelial cell; TFAP4, transcription factor activating enhancer binding protein 4; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor-α; TTR, time to recurrence; VEGF, vascular endothelial growth factor.

*HepG2Cyp2E1, cell line overexpressing ethanol-metabolizing enzyme cytochrome P450 2E1. †For differential expression in normal and HCC tissue by qRT-PCR in the 16 samples and for differential expression in normal and HCC tissue, and the remaining analysis with the 105 samples. ‡Sample data from different public databases without reporting total number of samples included in the study.
<table>
<thead>
<tr>
<th>Targeting strategy</th>
<th>Specifications</th>
<th>NRP</th>
<th>Method of measurement</th>
<th>Model</th>
<th>Sample type</th>
<th>Outcome</th>
<th>Study</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>shRNA silencing</td>
<td>NRP1 shRNA VSV-lentivirus</td>
<td>NRP1</td>
<td>RT-PCR</td>
<td>In vitro</td>
<td>Mahlavu, Huh-7, SK-Hep1 and HEK293T cell lines</td>
<td>Migration</td>
<td>Liao et al.</td>
<td>2008</td>
</tr>
<tr>
<td></td>
<td>NRP1 shRNA in lentivirus-based RNAi vector pLVTHM</td>
<td>NRP1</td>
<td>qRT-PCR, Western blot and ICC</td>
<td>In vitro</td>
<td>Human hepatoma-derived HCCLM6 cell line HCCLM6 xenograft nude mice</td>
<td>Proliferation</td>
<td>Xu and Xia</td>
<td>2013</td>
</tr>
<tr>
<td>Lentiviral-based NRP1 shRNA from Origene</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>In vitro</td>
<td>Bel-7402 and SMMC-7721 cell lines</td>
<td>Cell viability and apoptosis</td>
<td>Lin et al.</td>
<td>2018</td>
<td></td>
</tr>
<tr>
<td>Lentivirus pGCSIL-RFPshNRP1 self-constructed</td>
<td>NRP1</td>
<td>Western blot and ICC</td>
<td>In vitro</td>
<td>HepG2</td>
<td>Tumor progression and migration</td>
<td>Xu et al.</td>
<td>2017</td>
<td></td>
</tr>
<tr>
<td>NRP1 shRNA produced by GeneChem</td>
<td>NRP1</td>
<td>qRT-PCR and Western blot</td>
<td>In vitro</td>
<td>HepG2, HCCLM3 and Huh-7</td>
<td>CSC properties, proliferation, migration, EMT and metastasis</td>
<td>Li et al.</td>
<td>2021</td>
<td></td>
</tr>
<tr>
<td>siRNA silencing</td>
<td>siRNA targeting mouse NRP1 (ID #155679, Ambion)</td>
<td>NRP1</td>
<td>ICC</td>
<td>In vivo</td>
<td>Transgenic HCC C57BL/6 mice</td>
<td>Tumor progression and vascular remodeling</td>
<td>Bergé et al.</td>
<td>2010</td>
</tr>
<tr>
<td></td>
<td>ON-TARGETplus NRP1 siRNA</td>
<td>NRP1</td>
<td>Western blot</td>
<td>In vitro</td>
<td>Mouse Hepa129 cell line C3H mice with Hepa129-derived tumor</td>
<td>Proliferation, apoptosis, inflammation and migration</td>
<td>Raskopf et al.</td>
<td>2010</td>
</tr>
<tr>
<td></td>
<td>NRP1 siRNA from Bioneer with Effectene reagent</td>
<td>NRP1</td>
<td>Western blot</td>
<td>In vivo</td>
<td>HepG2 and Huh-7</td>
<td>Cell death</td>
<td>Lee et al.</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td>ON-TARGETplus NRP2 siRNA</td>
<td>NRP2</td>
<td>qRT-PCR and Western blot</td>
<td>In vitro</td>
<td>3sp, SNU-398, SNU-423, SNU-449, SNU-475, FLC-4 cell lines</td>
<td>Migration, mesenchymal properties, TGF-β signaling</td>
<td>Wittmann et al.</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td>NRP1 siRNA and lipofectamine 2000</td>
<td>NRP1</td>
<td>Western blot</td>
<td>In vitro</td>
<td>HepG2</td>
<td>Cell viability and migration</td>
<td>Lv et al.</td>
<td>2019</td>
</tr>
<tr>
<td></td>
<td>NRP1 siRNA from Qiagen</td>
<td>NRP1</td>
<td>Western blot</td>
<td>In vitro</td>
<td>HepG2</td>
<td>Cell proliferation</td>
<td>Arab et al.</td>
<td>2020</td>
</tr>
<tr>
<td>Targeting strategy</td>
<td>Specifications</td>
<td>NRPs</td>
<td>Method of measurement</td>
<td>Model</td>
<td>Sample type</td>
<td>Outcome</td>
<td>Study</td>
<td>Year</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------</td>
<td>------</td>
<td>------------------------</td>
<td>-------</td>
<td>-------------</td>
<td>---------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>TCDD</td>
<td>NRP 1</td>
<td>qRT-PCR</td>
<td>In vitro</td>
<td>HepG2</td>
<td>Xenobiotic toxicity</td>
<td>Lee et al.</td>
<td>2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NRP 1</td>
<td>qRT-PCR and Western blot</td>
<td>In vitro</td>
<td>HepG2, SK-HEP-1 and PLC/PRF/S cell lines</td>
<td>Cell proliferation, apoptosis and invasion</td>
<td>Bergé et al.</td>
<td>2011</td>
</tr>
<tr>
<td>miRNAs overexpression</td>
<td>miR-148b</td>
<td>NRP 1</td>
<td>IHC</td>
<td>In vivo</td>
<td>PLC/PRF/S xenograft BALB/c nude mice</td>
<td>Tumor progression, angiogenesis, microRNA modulation and CSC properties</td>
<td>Liu et al.</td>
<td>2015</td>
</tr>
<tr>
<td>miR-340-5p/miR-452-5p</td>
<td>NRP 1</td>
<td>qRT-PCR and Western blot</td>
<td>In vitro</td>
<td>Hep3B BALB/c athymic nude mice injected with Hep3B</td>
<td>microRNAs and circRNAs modulation</td>
<td>Yang</td>
<td>2020</td>
<td></td>
</tr>
<tr>
<td>Antibody</td>
<td>Truncated tissue factor anti-NRP1 monoclonal antibody</td>
<td>NRP 1</td>
<td>NR</td>
<td>In vivo</td>
<td>HepG2 xenograft BALB/c nude mice</td>
<td>Tumor progression</td>
<td>Xu et al.</td>
<td>2019</td>
</tr>
</tbody>
</table>

circRNA, circular RNA; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; HCC, hepatocellular carcinoma; ICC, immunocytochemistry; IHC, immunohistochemistry; miRNA, microRNA; NR, not reported; NRP, neuropilin; qRT-PCR, real-time reverse transcription polymerase chain reaction; shRNA, short hairpin RNA; sRNA, small interference RNA; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TGF-β, transforming growth factor β.
ings highlight the necessity of further studies to clarify NRP2’s exact role in HCC.

Early HCC diagnosis is one of the main objectives of current clinical studies, where identifying useful biomarkers is an exciting area. While all tissue and serum biomarkers are valuable tools for diagnosis at any tumor stage, non-invasive methods are usually preferred. Only two studies have analyzed the potential use of either NRP1 or NRP2 as serum biomarkers, reporting that NRP1 could be a useful protein for HCC diagnosis based on its elevated levels in serum samples from patients with advanced HCC.[17,23]

**NRP1 and NRP2: Prognostic Biomarkers**

Consistent with previous evidence, numerous studies have evaluated the prognostic role of NRP1 and NRP2 in HCC patients.[15,16,24-26,29,37] NRP1 overexpression was significantly correlated with shorter overall survival (OS) [16,24-26,29] and recurrence-free survival (RFS)[16,24] in patients with HCC. Moreover, NRP1 expression was correlated with lower OS and disease-free survival.[30] Furthermore, NRP1 expression was correlated with RAD51 expression, a marker of tumor progression.[31] Peritumoral NRP1 levels have also been evaluated in HCC human samples. Patients with higher peritumoral NRP1 expression had higher OS, higher time to recurrence, and lower early recurrence incidence.[32] Intriguingly, a serum signature of nine markers (PDGF-BB, Met, platelet-derived growth factor-BB, platelet-derived growth factor-A, platelet-derived growth factor-BB, and Met) correlated significantly with OS, progression-free survival, and early progression disease in advanced HCC.[33]

In addition, associations between NRP overexpression and other clinical characteristics related to tumor aggressiveness have been reported. Both NRPs correlated strongly with advanced HCC stages, age, sex, tumor size, and hepatitis B virus (HBV) infection.[16,24,30] Nevertheless, NRP1 overexpression was positively correlated with alpha-fetoprotein and other liver function markers and negatively correlated with albumin (Alb) and pre-Alb.[17] These findings highlight the necessity of further studies to clarify NRP2’s exact role in HCC.

**NRP effects on tumor progression and associated signaling pathways**

NRP1 and NRP2 promote tumor progression by modulating cell proliferation, viability, and apoptosis, with NRP1 being the most characterized.[34] Numerous preclinical investigations showed that NRP1 downregulation decreased the growth and viability of HepG2, SK-HEP-1, PLC/PRF/5, HC-CLM6, Huh-7, Bel-7402, SMMC-7721, and HCCLM3 cells. However, no effects were observed in mouse Hepa129 cells.[35] Similar effects have been described in animal models, where a marked tumor growth inhibition was observed after NRP1 knockdown.[12,16,24,26,29,37,42,44,49] A five-gene signature that included NRP1 was identified as an independent risk factor for a faster tumor growth rate in HCC patients.[41] Furthermore, an interaction between NRP1 and VEGFA promoted HCC tumorigenesis by dysregulating cell-to-cell interactions in patients with HCC.[42]

Similarly, apoptosis was altered when NRP1 expression was abolished in both *in vitro* and *in vivo* models, leading to an increase in cleaved caspase-3 levels and TUNEL-positive cells.[12] However, a previous study did not report cell death changes after NRP1 silencing in two HCC cell lines.[38] Peritumoral NRP1 expression was decreased and correlated with increased tumor size and other associated characteristics,[39] highlighting NRP1’s crucial role in HCC tumor progression. In addition, NRP1 correlated positively with the immune marker cluster of differentiation 36 (CD36), a potential prognostic and immunologic marker in different cancers, including HCC.[43] Intriguingly, a recent study treated HepG2 cells with the supernatant from NRP1-downregulated HSCs, causing a decrease in lipid droplet content and insulin-like growth factor binding protein-3 (IGFBP3) expression, and an increase in serpin fami-
Figure 2. Main cellular and molecular mechanisms modulated by NRP1 and NRP2. NRPs are expressed in tumor cells and other tumor-associated populations that constitute the tumor microenvironment and participate in the immune response. Both NRP1 and NRP2 are expressed in a broad number of cell types and are involved in different cellular and molecular mechanisms responsible for HCC development and progression, modulating several cellular processes. EMT, epithelial-to-mesenchymal transition; IFN-β, interferon beta; IFN-γ, interferon gamma; IL-10, interleukin-10; NRP, neuropilin; TGF-β, transforming growth factor β; TNF-α, tumoral necrosis factor-α.
ly A member 12 (SERPINA12) levels.43

Only one study has analyzed the processes modulated by NRP2.35 Specifically, it was directly modulated by TGF-β signaling and correlated with a mesenchymal-like phenotype in vitro. Curiously, while TGF-β overexpression increased NRP2 levels, NRP2 silencing did not exhibit significantly altered TGF-β expression.35

Role of NRP in migration and invasion-related processes

Invasion and migration processes are well-described events that characterize the aggressiveness of solid tumors and are highly associated with tumor progression and recurrence.50

The NRPs’ main effects in human pathologies are generally related to the modulation of signaling pathways that drive angiogenesis and cancer cell migration.36 Numerous studies evaluated the NRPs’ role in these cellular processes in preclinical models12,26,35-37,39,40,42,43 and human patients (Fig. 2).24,25 NRP2 downregulation significantly decreased the migration of two different HCC cell lines.35 However, most studies have described similar results after NRP1 knockdown, finding lower cell migration ability in different cellular models, including the HCCLM3,26,34 Huh-7,26,31,32 HepG2,40,42 Mahlavu, SK-Hep1, and HEK293T human lines37 and mouse Hepa129 cell line.37

NRP1 was also overexpressed in the highly metastatic Bel-7402 cell line, but was underexpressed in the less metastatic HepG2 and SMMC-7721 cell lines.16 A recent study evaluated NRP1 function in metastasis, generating an HCC mouse model using NRP1-silenced and non-silenced HCCLM3 cells.26 Curiously, they found that NRP1 downregulation greatly diminished the number of grafted mice with lung metastasis (1 of 5), compared to control mice (5 of 5).26 Similar findings have been reported in studies on HCC patients, where those with elevated NRP1 levels had a higher probability of venous invasion and metastasis.24,25

At the molecular level, the NRPs’ role in angiogenesis-related signaling has been analyzed by two studies.26,33 After VEGF silencing in the mouse Hepa129 HCC cell line, both NRP1 and NRP2 levels were markedly downregulated,33 supporting their interplay with the VEGF family ligands described in other tumor types.10 Moreover, a recent study found that NRP1 modulated the epithelial-to-mesenchymal transition (EMT) pathway in two HCC cell lines, with decreased N-cadherin and vimentin expression and increased E-cadherin levels observed after NRP1 silencing.26

Other relevant clinical aspects associated with tumor invasion and metastasis have been evaluated and associated with NRPs (Fig. 2).12,36,37,39,40 Two independent studies performed by the same research group successfully inhibited NRP1 activity through genetic silencing or inhibitor treatment, significantly reducing tumor vascular remodeling.12,36 Moreover, an in vitro matrigel assay analysis of capillary-like structures showed that NRP1 inhibition with peptide N decreased the formation of capillary-like structures and other tumor-associated characteristics.37 In this line, while the tube-forming ability of mouse Hepa129 cells decreased after NRP1 silencing,37 NRP1 overexpression via microRNA (miRNA)-148b (miR-148b) silencing raised the tube-forming ability of human PLC/PRF/5 HCC cells.40 Moreover, lower neovascularization was observed in an in vivo HCC mouse model after NRP1 silencing.39

Studies on human HCC patients have described similar results. NRP1 overexpression was significantly correlated with intrahepatic metastasis35 and vascular invasion24,26 in two independent HCC cohorts, supporting the potential roles for NRP1 and NRP2 in the mechanisms underlying HCC progression.

NRPs and the immune response

HCC is a complex and heterogeneous tumor in which malignant hepatocytes and other tumor-associated cells affect the development, progression, and drug responsiveness of tumor cells.55,56 Both innate and adaptive immune cells have been strongly associated with the modulation of cellular responses to chronic inflammation, fibrosis, or cirrhosis contributing to hepatocarcinogenesis and HCC progression.56 Several investigations have focused on the tumor immunological microenvironment as a key mechanism in HCC (Fig. 2).34,36,37,57-61

A preclinical study exploring NRP1 and NRP2 in Mdr2 deficient HCC mice, found their expression higher in macrophages than in hepatocytes.57 Additionally, thymocytes isolated from mice bearing a transplantable hepatoma 22a had higher NRP1 levels than control animals.18 Among liver immune cells, dendritic cells expressed NRP1, correlating with RAD51 and transcription factor activating enhancer binding protein 4 (TFAP4),59 valuable prognosis and immune response markers, respectively, in patients with HCC.34,59
Association between NRPs and the tumor microenvironment

The tumor microenvironment is a key factor in cancer development and progression that undergoes dynamic changes involving various components. Cancer stem cells (CSCs), HSCs, cancer-associated fibroblasts (CAFs), cytokines and growth factors are tumor microenvironment components strongly associated with HCC progression. Numerous studies have assessed the modulatory effects of the tumor microenvironment on NRP expression and activity, recently reporting some exciting findings.

CSCs are a small population of tumor cells that control differentiation, tumorigenicity, metastasis, and therapeutic resistance in HCC, with NRP1 appearing to play a key role. Specifically, NRP1 downregulation in an in vitro HCC model significantly decreased the liver CSC population. In contrast, NRP1 was overexpressed in the CSC population of two HCC cell lines and expressed explicitly in CAFs and tumor-associated endothelial cells in HCC patients. Among the cell types influencing the tumor microenvironment, HSCs have been broadly associated with HCC progression. However, only one study evaluated NRP1 expression in HSCs, finding increased HepG2 cell proliferation in matrix conditioned with HSCs overexpressing NRP1.

Low oxygen conditions, hypoxia, are crucial in HCC development, progression and chemoresistance. A hypoxic microenvironment modulated both NRPs in vitro, decreasing NRP1 levels but increasing NRP2 levels. Intriguingly, this study found that hypoxia increased NRP expression when VEGF was silenced. Similarly, cobalt chloride-induced hypoxia decreased NRP1 expression in the liver L02 cells. Moreover, hypoxia directly modulated NRP1 in an in vivo HCC model, with NRP1 levels decreased in peritumoral tissue and negatively correlated with hypoxia-inducible factor 1-alpha (HIF-1α) levels. Overall, both NRPs appear to contribute to tumor microenvironment modulation, showing key interactions with different tumor-associated cell types and the oxygen conditions in HCC.

DISCUSSION

While NRPs were described as key proteins in the nervous system through axon guidance modulation, recent findings...
also indicate an intriguing role in HCC and other cancer types.\textsuperscript{7,8}

Numerous studies have indicated a potential role for NRP1 as a tumor biomarker in HCC,\textsuperscript{12,14,16-20,23,24,26} while fewer and contradictory results have been reported for NRP2.\textsuperscript{13,21,28} Studies have found both NRPs overexpressed in tumor samples from different cancer types, including cholangiocarcinoma,\textsuperscript{18,71-75} supporting mainly NRP1, but also NRP2, as potential biomarkers in HCC. However, further studies are needed to clarify their exact role and improve the diagnostic tools available for human HCC. Moreover, both NRPs are strongly associated with worse prognoses and different tumor-associated parameters in HCC patients.\textsuperscript{15,16,24-26,29-33,35} Similar findings were found with different solid tumors, where NRPs negatively correlated with prognosis and higher invasion and metastasis risk,\textsuperscript{18,71,73-75} highlighting their potential role as diagnostic tools for HCC patient prognosis.

Most studies evaluating NRP function in HCC have used preclinical models, where mainly NRP1, but also NRP2, exhibited an interesting modulatory roles by promoting cell survival, tumor progression, invasion and migration, and inhibiting apoptosis.\textsuperscript{12,15,17,26,35-48,51-54} Similar findings were reported for other tumor types, describing TGF-β and other signaling pathways as potential targets of NRP regulation.\textsuperscript{76-79} Additionally, invasion, migration and metastasis were also found to be strongly modulated by both NRPs in different solid tumor models,\textsuperscript{24,80,81} supporting roles for NRP1 and NRP2 in mechanisms underlying tumor progression and spread. Nevertheless, while these findings highlight interesting function for NRPs in HCC tumor progression and invasion abilities, further studies are needed to identify the exact mechanisms and signaling pathways modulated by NRP1 and NRP2.

Based on solid tumor heterogeneity and multiple processes involved in tumor cell adaptation and progression, numerous studies have described key functions for NRP1, and to a lesser extent NRP2, in modulating the immune response against tumor hepatocytes,\textsuperscript{24,36,37,57-61} that are associated with the potential interplay between NRPs, miRNAs,\textsuperscript{40,51,64,65} and the tumor microenvironment.\textsuperscript{15,26,29,33,40,53} Other studies have also reported a correlation between NRP1\textsuperscript{82-85} and NRP2\textsuperscript{86} expression in different immune cells and immune response suppression in other cancers.\textsuperscript{82-86} Specifically, NRPs had a crucial role in modulating the immune response,\textsuperscript{10,82} where NRP1 appeared to be associated with immune response suppression in cancer.\textsuperscript{82} Regulatory T cells with depleted NRP1 exhibited decreased function, restoring the antitumor immunity and TNF-α production.\textsuperscript{83} Moreover, NRP1 expression in macrophages, dendritic cells and other associated cell populations was associated with a restrained inflammatory response.\textsuperscript{84,85} NRP2 expression in tumor-associated macrophages promoted tumor growth by regulating macrophage phagocytosis.\textsuperscript{86} Therefore, based on these findings in different HCC models, both NRPs potentially play a key role in modulating the tumor-associated immune response, making them potential biomarkers in the HCC tumor landscape.

Consistent with growing evidence reinforcing the key role of miRNAs in cancer, these non-coding RNAs modulate NRP1 in HCC, increasing tumor hepatocyte proliferation and migration.\textsuperscript{60,71,64,65} Different miRNAs (e.g., miR-376a) or circRNAs (e.g., circ-LDLRAD3) regulated tumor progression in other cancer models.\textsuperscript{76,78} While few studies have provided clearer results on the mechanisms underlying the potential interplay between miRNAs and NRPs, these findings suggest that miRNAs could be potential modulators of NRPs expression and activity, but mainly of NRP1, controlling key processes involved in HCC progression and invasion.

Several investigations have described that NRPs, primarily NRP1, are highly influenced by the tumor microenvironment, with tumor-associated cell populations playing a crucial role.\textsuperscript{87-89} Among them, CAFs and CSCs could be modulated by NRP1 or NRP2 in different cancer types.\textsuperscript{87-89} Indeed, NRP1 appears to have an interesting role in the response of different cell types in the tumor microenvironment, acting as a potential modulator of tumor adaptation and progression. Moreover, the interplay between hypoxia and NRPs has recently been explored in other cancers.\textsuperscript{90-92} However, these results showed an opposite hypoxia effect to HCC, with increased NRP1\textsuperscript{93} but decreased NRP2 expression under hypoxic conditions.\textsuperscript{93} Together with the studies on HCC, these results indicate that further investigations are needed to obtain a clearer understanding of the exact mechanism through which hypoxia and NRPs might contribute to tumor development.

Limitations

This review aimed to provide a clear and complete understanding of the main mechanisms modulated by NRPs in HCC development and progression. Nevertheless, some limitations exist that are mostly associated to the high heterogeneity among studies. The main limitation was that most articles
examined only one NRP, with 40 articles focused on NRP1 but only four on NRP2; five examined both NRPs. This limitation led to greater discordance in the results obtained, mainly for NRP2, increasing the uncertainty of the conclusions drawn. Moreover, as shown in Table 1A, the methods employed for determining NRP1 or NRP2 levels were inconsistent, with most articles focusing on one NRP, using different targeting strategies. Discrepancies between studies could be explained by the different methods used to measure NRPs and the chosen targeting strategy.

Additionally, while multiple cellular and molecular processes had been evaluated, the number of studies analyzing each mechanism was highly heterogeneous. The diagnostic and prognostic values of NRP1 and NPR2, and their crucial role in invasion and migration, were the main processes studied, with few articles focusing on their interplay with miRNAs or the tumor microenvironment. While these interactions are key mechanisms in cancer development and progression, only five and six studies have explored them in HCC, respectively.

Finally, although increasing numbers of human studies have been published, they have not always described the main characteristics of HCC patients, with etiology, age, or country missing in some articles. Moreover, public databases hindered data extraction by not stating the number of patients included in the analysis. In summary, some important limitations should be considered when understanding and interpreting the main conclusions of this systematic review.

**Figure 3.** Main findings from the studies included in this systematic review describing modulatory effects associated to NRP1 and NRP2 in HCC. Specific modulatory effects exerted by both NRPs are graphically shown, together with correlations observed in different cellular processes and molecular mechanisms. α-SMA, α smooth muscle actin; CSC, cancer stem cell; DFS, disease-free survival; IFN-β, interferon beta; IFN-γ, interferon gamma; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; TFAP4, transcription factor activating enhancer binding protein 4; TGF-β, transforming growth factor beta; TNF-α, tumoral necrosis factor-α; VEGF, vascular endothelial growth factor.
CONCLUSIONS AND FUTURE PERSPECTIVES

To the best of our knowledge, this article is the first systematic review focusing on the role of NRPs in HCC, summarizing all the results obtained from preclinical and clinical studies (Fig. 3). Increasing evidence suggests vital roles for these receptors (NRP1 and NRP2) in tumor-associated processes. The results summarized here suggest that NRP1 could act as a potential diagnostic biomarker and, with NRP2, an interesting prognostic biomarker in HCC patients. The NRPs have modulatory effects on different signaling pathways that promote tumor progression and are crucial mediators of the HCC cell invasion and migration abilities. The tumor-associated immune response is also strongly associated with NRPs, mainly NRP1, and the tumor microenvironment, in which different tumor cell populations have higher NRP1 levels. The interplay between miRNAs and NRPs has gained interest since several miRNAs directly modulate NRP1, restraining tumor cell proliferation. In summary, NRPs appear to have critical roles in various processes involved in tumor development and progression, suggesting the potential of both, but mainly NRP1, as tumor biomarkers and potential targets for improving the HCC patient outcomes.

Authors’ contribution
All authors were responsible for study conception and design, interpretation of the data, and drafting of the manuscript. Systematic literature review, data extraction, and data analysis were performed by P.F.-P., T.P.-S., C.M.-B. and B.S.-M. In addition, M.J.T., J.G.-G. and J.L.M. supervised the study, and carried out the review and editing of the paper. The final version of the manuscript was approved by all authors.

Acknowledgements
This work was supported by the Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) [project PID2020-119164RB-I00]. CIBerehd is funded by Instituto de Salud Carlos III (ISCIII), Spain. P.F.-P. is supported by the Ministry of Education (MCIN/AEI/10.13039/501100011033) [grant FPU17/01995] and T.P.-S. by the Asociación Española Contra el Cáncer (AECC)-Junta Provincial de León, Spain.

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

49. Li Z, Bao H. Deciphering key regulators of Inonotus hispidus petroleum ether extract involved in anti-tumor through whole transcriptome and proteome analysis in H22 tumor-bearing mice model. J Ethnopharmacol 2022;296:115468.

Neuropilins in hepatocellular carcinoma
Paula Fernández-Palanca, et al.

http://www.e-cmh.org
https://doi.org/10.3350/cmh.2022.0425

317


87. Glinka Y, Mohammed N, Subramanian V, Jothy S, Prud’homme
GJ. Neuropilin-1 is expressed by breast cancer stem-like cells and is linked to NF-κB activation and tumor sphere formation. Biochem Biophys Res Commun 2012;425:775-780.


Chronic hepatitis B with concurrent metabolic dysfunction-associated fatty liver disease: Challenges and perspectives

Shang-Chin Huang and Chun-Jen Liu

Department of Internal Medicine, National Taiwan University Hospital Bei-Hu Branch, Taipei; Division of Gastroenterology and Hepatology, Department of Internal Medicine, National Taiwan University Hospital, Taipei; Hepatitis Research Center, National Taiwan University Hospital, Taipei; Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

The prevalence of metabolic dysfunction-associated fatty liver disease (MAFLD) has increased among the general population and chronic hepatitis B (CHB) patients worldwide. Although fatty liver disease is a well-known risk factor for adverse liver outcomes like cirrhosis and hepatocellular carcinoma, its interactions with the hepatitis B virus (HBV) and clinical impacts seem complex. The presence of hepatic steatosis may suppress HBV viral activity, potentially leading to attenuated liver injury. In contrast, the associated co-morbidities like diabetes mellitus or obesity may increase the risk of developing adverse liver outcomes. These findings implicate that components of MAFLD may have diverse effects on the clinical manifestations of CHB. To this end, a clinical strategy is proposed for managing patients with concurrent CHB and MAFLD. This review article discusses the updated evidence regarding disease prevalence, interactions between steatosis and HBV, clinical impacts, and management strategies, aiming at optimizing holistic health care in the CHB population. (Clin Mol Hepatol 2023;29:320-331)

Keywords: Fatty liver; Hepatitis B virus; Non-alcoholic fatty liver disease; Metabolic syndrome; Hepatocellular carcinoma

INTRODUCTION

Hepatic steatosis: an emerging global health issue

Fatty liver is the hepatic manifestation of systemic metabolic dysregulation and has become an emerging etiology for cirrhosis and hepatocellular carcinoma (HCC) worldwide. It is estimated that nearly a third of people are affected by fatty liver diseases. Moreover, the estimated prevalence is increasing in Asian countries, from 25.3% between 1999 and 2005 to 33.9% between 2012 and 2017. As a result, the optimal strategy for the diagnosis and management of fatty liver diseases is of top priority at the global public health level.

New concept and nomenclature: metabolic dysfunction-associated fatty liver disease (MAFLD)

In 2020, a new definition for fatty liver disease, MAFLD, was...
proposed in an expert consensus meeting.\(^3\) Compared with the traditional definition of non-alcoholic fatty liver disease (NAFLD), the new criteria of MAFLD do not need to exclude patients with chronic viral hepatitis, excessive alcohol intake, medication-related steatosis, or other chronic liver diseases; instead, the diagnosis of MAFLD is based on the presence of hepatic steatosis, plus one of the following three clinical situations: overweight/obesity, type 2 diabetes mellitus (DM), or two metabolic risk factors (Fig. 1).\(^3\) The evolution of the definition makes the clinical diagnosis easier and has been shown to include more patients with higher disease severity.\(^4\)\(^-\)\(^8\) Particularly, unlike NAFLD, the diagnosis of MAFLD can be made for patients with other concurrent chronic liver diseases, including chronic hepatitis B (CHB).\(^9\)

**Concurrent MAFLD in the hepatitis B population**

Although a lower prevalence of hepatic steatosis in CHB patients than that in the general population has been reported,\(^10\) co-existing fatty liver disease among the CHB population is frequently seen in HBV endemic areas. According to a prior meta-analysis of 17 studies, the prevalence of fatty liver was about 29.6% in patients with CHB;\(^11\) in another meta-analysis of 54 studies with 28,648 CHB patients, the pooled prevalence of hepatic steatosis is up to 32.8%\(^12\); a more recent meta-analysis of 98 studies with 48,472 patients demonstrated an even higher global prevalence of 34.93%.\(^13\) Clinical manifestations, reciprocal interaction, and impacts are essential issues to be addressed. This review article will focus

**Figure 1.** Disease definition of MAFLD. The new criteria do not need to exclude patients with other concomitant liver diseases or alcohol intake. MAFLD, metabolic dysfunction-associated fatty liver disease; DM, diabetes mellitus; TG, triglycerides; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance index; CRP, C-reactive protein.

**Abbreviations:**

MAFLD, metabolic dysfunction-associated fatty liver disease; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; CAP, controlled attenuation parameter; HBcAg, hepatitis B core antigen; siRNA, small interfering RNA; NASH, non-alcoholic steatohepatitis; PSM, propensity score matching; OR, odds ratio; CI, confidence interval; HR, hazard ratio; AUROC, area under receiver operating characteristics curve; NA, nucleotide analogue; ALT, alanine aminotransferase; GLP-1, glucagon-like peptide 1; LSM, liver stiffness measurements; HDL-C, high-density lipoprotein cholesterol; CT, computed tomography; MRI, magnetic resonance imaging

http://www.e-cmh.org  
https://doi.org/10.3350/cmh.2022.0422
on the interactions between MAFLD and CHB, as well as the management strategies for CHB patients with co-existing MAFLD.

**INTERACTION AND IMPACTS**

**Inverse correlation between steatosis and HBV activity**

Regarding the epidemiology, as aforementioned, a lower prevalence and incidence of steatosis in patients with CHB than in the general population has been consistently reported in several studies; in addition, higher levels of serum HBV DNA were associated with a lower prevalence of fatty liver among patients with CHB. On the other hand, CHB patients with concurrent steatosis tended to have lower viral activity, including lower proportions of hepatitis B e antigen (HBeAg) positivity and lower serum HBV DNA levels, as well as higher rates of hepatitis B surface antigen (HBsAg) seroclearance. In a study enrolling 506 untreated CHB patients, the level of HBV viral load was lower in those with fatty liver than in those without fatty liver in a dose-dependent manner based on controlled attenuation parameter (CAP) value. In a study of 3,212 untreated CHB patients, the proportions of serum HBeAg positivity, HBV viremia, intrahepatic HBsAg and hepatitis B core antigen (HBcAg) positive staining on liver tissue were fewer in those with steatosis. Similarly, the inverse correlation between hepatic steatosis and HBV viral activity was confirmed in a recent meta-analysis.

The underlying mechanisms for the negative association between hepatic steatosis and HBV viral activity have been explored in animal and cellular models. The hepatic steatosis in an HBV-immunocompetent mouse model fed with high-fat diets significantly attenuated the levels of serum HBeAg, HBsAg, HBcAg, and HBV DNA. In the in vitro model, steatosis inhibited HBsAg and HBV DNA secretion by the induction of endoplasmic reticulum stress in hepatocytes. Adiponectin which suppresses hepatic steatosis was found to be a potentially important mediator; a study using the in vitro model of HepG2-hepatitis B virus-stable cells demonstrated that the viral replication was upregulated by adiponectin and was downregulated by the small interfering RNAs (siRNAs) for adiponectin; this finding was consistent with a prospective study of 266 CHB patients, which showed that the levels of adiponectin increased in those with higher HBV viral load. Of note, although the above mechanistic findings partially explained the viral suppression in CHB patients with concurrent fatty liver disease, current understandings remain only the tip of the iceberg.

**Uncertain association between fatty liver disease and fibrosis**

MAFLD is a disease with a broad spectrum from simple steatosis to steatohepatitis, and the latter may cause inflammation as well as liver fibrosis with resultant cirrhosis. In the general population, MAFLD is a known etiology for cirrhosis; however, whether concurrent MAFLD among CHB patients will aggravate fibrosis progression is inconclusive. In two studies using FibroScan™ to define fatty liver disease in CHB patients, hepatic fibrosis was positively associated with the CAP value. In a retrospective study of 1,089 CHB patients with liver histological evaluation, patients with concurrent non-alcoholic steatohepatitis (NASH) had a higher degree of liver fibrosis; consistently, steatosis was associated with fibrosis and cirrhosis in another biopsy-proven cohort of 270 CHB patients. However, a large retrospective cohort study enrolling 6,786 CHB patients demonstrated a lower incidence of cirrhosis in those with fatty liver than those without, either before or after propensity score matching (PSM); the 10-year cumulative incidence was 10.5% vs. 15.5%, respectively, in the PSM cohort. A meta-analysis evaluating 6,232 CHB patients from 20 studies with available histology or transient elastography data showed no association between steatosis and fibrosis (pooled odds ratio 0.87, 95% confidence interval [CI] 0.54–1.39); a similar result was also demonstrated in another meta-analysis. Collectively, the exact impact of MAFLD on liver fibrosis among CHB patients remains uncertain, and this may be partially attributable to the different severity of fatty liver disease in each study population, leading to a variable degree of liver injury and resultant fibrosis.

**Inconclusive results for MAFLD and risk of HBV-related HCC**

HCC development is one of the major adverse outcomes in patients with chronic liver diseases, including MAFLD. According to a large cohort study, the annual incidence of HCC in patients with NAFLD was 0.021%, 10-fold higher than
those without liver disease.\textsuperscript{30} However, the influence of co-existing steatosis in CHB patients remained controversial among studies (Table 1). Although MAFLD and CHB are well-established etiologies for HCC, whether concurrent MAFLD and CHB lead to a higher risk of HCC development than CHB alone is inconclusive, according to current evidence. In the prospective cohort studies with more than two-thousand male CHB patients in Taiwan, fatty liver at baseline was an independent protective factor for HCC development.\textsuperscript{20,31} Likewise, another cohort study of 6,786 CHB patients showed a reduced 10-year risk of HCC in those with steatosis than those without steatosis, 3.74% versus 6.18%, respectively; the protective effect of steatosis remained unchanged after PSM.\textsuperscript{29} In two recent studies conducted in Hong Kong and South Korea quantifying the degree of steatosis by FibroScan\textsuperscript{TM}, a higher CAP value was associated with a lower risk of HCC occurrence in CHB populations.\textsuperscript{32,33} Nevertheless, other studies enrolling CHB patients receiving liver biopsies demonstrated the opposite impact on HCC risk. A retrospective cohort study on a liver biopsy cohort of 270 CHB patients showed concurrent fatty liver was an independent risk factor of HCC (adjusted hazard ratio [HR] 7.27, 95% CI 1.52–34.76, P=0.013);\textsuperscript{28} another study of 1,089 CHB patients with available liver histology found NASH was independently associated with a higher risk of HCC;\textsuperscript{27} recently, the same cohort using the new criteria of MAFLD defined by histology revealed MAFLD was associated with poorer HCC-free survival (adjusted HR 1.93, 95% CI 1.17–3.21); however, steatohepatitis did not increase the risk of HCC among patients with MAFLD, indicating metabolic dysfunction rather than steatosis per se as the key role in the hepatocarcinogenesis.\textsuperscript{34} A recent meta-analysis showed that the presence of fatty liver, especially biopsy-proven steatosis, was associated with an increased risk of HCC in CHB patients.\textsuperscript{21}

One of the plausible explanations for the above conflicting results may be the heterogeneous study populations enrolled in each study; CHB patients fulfilling the indication of the liver biopsy were expected to have higher disease severity and represented a minority among the broad disease spectrum of CHB and MAFLD, leading to the diverse results. This speculation was supported by a meta-analysis that showed no significant association between steatosis and HCC after excluding those with biopsy-proven fatty liver.\textsuperscript{21} Another factor is the influence of the co-existing metabolic dysfunction in patients with MAFLD, including obesity or DM, which are also the established risk factors for HCC occurrence in CHB.\textsuperscript{35,37} In other words, the simple steatosis and metabolic dysfunction required for diagnosing MAFLD may have diverse effects on hepatic carcinogenesis exclusively in CHB patients (Fig. 2).\textsuperscript{38} Therefore, strategies for optimal risk stratification and individualized management for those with concurrent MAFLD need to be developed in future studies.

**MANAGEMENT STRATEGIES**

**CAP for evaluation of steatosis and steatohepatitis in CHB**

Liver biopsy is the gold standard for the diagnosis of hepatic steatosis; however, the risk of internal bleeding is the primary concern in clinical practice.\textsuperscript{39} Instead, non-invasive approaches are developed for the evaluation of fatty liver disease. Although the magnetic resonance imaging proton density fat fraction has the best accuracy among the non-invasive methods,\textsuperscript{40,41} CAP by FibroScan\textsuperscript{TM} (Echosens \textsuperscript{TM}, Paris, France) is the point-of-care technique for the measurement of attenuation during ultrasonography to estimate the degree of steatosis with the advantages of relatively low cost and requirement in first-line clinical settings,\textsuperscript{42} and it has also been validated in patients with CHB. In a study of 366 treatment-naive CHB patients receiving liver biopsy, the accuracy of CAP for steatosis was better than those of hepatic steatosis index and ultrasonography, with the area under receiver operating characteristics curve (AUROC) up to 0.932 for histology S ≥2, although a higher overestimation rate (30.5%) was also found.\textsuperscript{43} In another study of 65 concurrent CHB-NAFLD patients receiving liver biopsy, including 34 with NASH and 31 without NASH, the serum levels of CK-18 M30, fasting glucose, HBV DNA, and CAP were the independent predictors for NASH, and the AUROC of combining above markers reached 0.961 with a sensitivity of 100% and specificity of 80.6%.\textsuperscript{44} The usage of CAP for evaluation of steatosis is common in the CHB population; however, the performance of related modalities like the FibroScan-aspartate aminotransferase score, which predicts high-risk population in NAFLD, is uncertain in CHB patients;\textsuperscript{35} in addition, comprehensive investigations on the association of CAP with long-term outcomes in longitudinal CHB cohorts are still to be explored.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Study population</th>
<th>Diagnosis of fatty liver diseases</th>
<th>Findings (Impact on HCC risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi et al.</td>
<td>Retrospectively enrolled 1,089 CHB patients with available biopsy data from 2 tertiary centers in Canada and the Netherlands*</td>
<td>Liver histology</td>
<td>Steatosis and NASH were associated with a higher risk</td>
</tr>
<tr>
<td>Chan et al.</td>
<td>Retrospectively enrolled 270 CHB patients with available biopsy data in a single center in Hong Kong</td>
<td>Liver histology</td>
<td>Fatty liver was associated with a higher risk (aHR 7.27, 95% CI 1.52–34.76)</td>
</tr>
<tr>
<td>Oh et al.</td>
<td>Retrospectively collected 1,823 CHB patients on NA from two centers in South Korea</td>
<td>CAP from FibroScan™ (≥222 dB/m)</td>
<td>A higher CAP value was associated with a lower risk in those with stiffness &gt;10 kPa (aHR 0.47, 95% CI 0.29–0.77)</td>
</tr>
<tr>
<td>Mak et al.</td>
<td>Prospectively enrolled 2,403 CHB patients from a single center in Hong Kong</td>
<td>CAP from FibroScan™ (≥248 dB/m)</td>
<td>A higher CAP value was associated with a lower risk (aHR 0.994 per dB/m, 95% CI 0.989–0.999)</td>
</tr>
<tr>
<td>Li et al.</td>
<td>Retrospectively collected 6,786 CHB patients from the United States and Taiwan</td>
<td>Ultrasonography or CT</td>
<td>Fatty liver was associated with a lower risk in anti-viral treated patients after PSM (HR 0.21, 95% CI 0.09–0.51)</td>
</tr>
<tr>
<td>van Kleef et al.</td>
<td>Retrospectively enrolled 1,076 CHB patients with available biopsy data from 2 tertiary centers in Canada and the Netherlands*</td>
<td>Liver histology</td>
<td>MAFLD was associated with a higher risk (aHR 1.93, 95% CI 1.17–3.21), but steatohepatitis was not a risk factor within MAFLD patients</td>
</tr>
<tr>
<td>Hsueh et al.</td>
<td>Prospectively enrolled 2,385 HBsAg-positive male civil servants in Taiwan</td>
<td>Ultrasonography</td>
<td>Steatosis was associated with a lower risk (sHR 0.49, 95% CI 0.36–0.66)</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; CHB, chronic hepatitis B; NASH, non-alcoholic steatohepatitis; NA, nucleoside/nucleotide analogue; CAP, Controlled Attenuation Parameter; HR, hazard ratio; aHR, adjusted hazard ratio; sHR, sub-distribution hazard ratio; CI, confidence interval; CT, computed tomography; PSM, propensity-score matching; HBsAg, hepatitis B surface antigen; MAFLD, metabolic dysfunction-associated fatty liver disease.

*The two studies were based on the same cohort.
Anti-viral treatment for HBV with concurrent MAFLD

CHB is an infectious disease without effective curable treatment thus far, although the nucleot(s)ide analogues (NAs) can suppress the viral replication in patients with high viral activity. Similar treatment initiation and monitoring strategies have been proposed according to current guidelines in patients with concurrent MAFLD. However, the presence of NASH may influence the clinical assessment of viral activity and liver enzymes. In addition, some studies revealed the potential adverse impact of concurrent steatosis on the treatment efficacy using NAs (Table 2). CHB patients with hepatic steatosis receiving entecavir were found to have lower rates of serum HBV DNA undetectability and alanine aminotransferase (ALT) normalization compared to those without steatosis.46,47 These findings were in line with two meta-analyses that showed poorer treatment responses in patients with concurrent fatty liver.13,48 In contrast, other studies showed comparable anti-viral treatment responses regardless of steatosis.49-51 Clinicians should pay attention to the possible interference by the concurrent hepatic steatosis since the virologic treatment response is highly associated with the long-term risk of HBV-related disease progression, including the development of HCC.52 In patients with concurrent MAFLD, especially those with steatohepatitis, the threshold for initiation and selection of NAs should be individually evaluated; we recommend a more aggressive strategy (a lower threshold) with high-potency NAs (like tenofovir alafenamide or entecavir) for this subpopulation. For those undergoing anti-viral agents, monitoring of serum ALT and HBV DNA levels and timely intervention for the poor responders are the keys to improving the prognosis in patients with concurrent MAFLD.

Prompt intervention for concurrent MAFLD in CHB

Despite the potential long-term protective effect of hepatic steatosis for HCC development in CHB patients, fatty liver is not permissive from the perspective of holistic medicine. In a cohort study of 7,761 patients using the Third National Health and Nutrition Examination Survey in the United States, those with MAFLD had a higher risk of all-cause mortality (HR 1.17,
CHB, chronic hepatitis B; NA, nucleoside/nucleotide analogue; CAP, Controlled Attenuation Parameter; ALT, alanine aminotransferase; CT, computed tomography; MRI, magnetic resonance imaging; HBeAg, hepatitis B e antigen; NAFLD, non-alcoholic fatty liver disease.

NAFLD was not associated with ALT normalization
or virological response.
Ultrasonography, CT, MRI, or liver histology
Retrospectively enrolled 555 CHB patients
receiving NAs in the United States
Li et al.51

Steatosis was not associated with virological
response or HBeAg seroclearance.
Liver histology
Retrospectively enrolled 196 HBeAg-positive
CHB patients receiving liver biopsy and NA
monotherapy in Taiwan
Chen et al.50

Steatosis or steatohepatitis was not associated
with ALT normalization or virological response.
Liver histology
Charatcharoenwitthaya et al.49 Prospectively enrolled 79 CHB patients
receiving liver biopsy and NAs in Thailand

Steatosis was associated with lower rates of ALT
normalization and virological response.
A meta-analysis of 7 and 9 studies with patients Liver histology, CAP from FibroScanTM, or
receiving antiviral therapy
ultrasonography
Jiang et al.13

Steatosis was associated with lower rates of ALT
normalization and HBV DNA clearance.
CAP from FibroScanTM
(≥224 dB/m)
Prospectively enrolled 153 CHB patients
receiving Entecavir in China
Chen et al.47

Steatosis was associated with lower rates of
antiviral responses and ALT normalization.
Ultrasonography
Prospectively enrolled 267 CHB patients
receiving Entecavir in China
Jin et al.

Definition of fatty liver diseases
Study population

326

46

Author(s)

Table 2. Influence of concurrent steatosis on the treatment efficacy of nucleot(s)ide analogues in patients with CHB

Findings

Clinical and Molecular Hepatology
Volume_29 Number_2 April 2023

95% CI 1.04–1.32);6 the presence of MAFLD was also associated with increased risks of cardiovascular diseases,6,53 chronic
kidney disease,54,55 and incident extrahepatic cancers.56 As a
result, active intervention for concurrent MAFLD is similarly
essential for the CHB population (Fig. 3).
Lifestyle modifications, including enhancing exercise and
diet control, are the core of effective therapy, and body
weight reduction is the goal and indicator for any intervention.57 According to current evidence, weight loss of 5–10%
by a hypocaloric diet (1,200–1,500 kcal per day), avoidance of
alcohol, fructose, saturated fatty acid or ultra-processed
foods, and regular exercise (either aerobic or resistance training) are practical approaches in daily practice.57-60 Although
direct evidence from prospective studies to confirm the efficacy of lifestyle modification in CHB patients with concurrent
MAFLD is lacking, patient education about the above points
is still recommended due to the significant benefits proven
in the general population.
The standard pharmacological therapy for steatohepatitis
has not been established yet, but several promising agents
are now in clinical trials. Semaglutide, one of the glucagonlike peptide 1 (GLP-1) agonists, showed its superiority in
NASH resolution over placebo in a 72-week, double-blind
phase 2 trial enrolling patients with histology-confirmed
NASH and fibrosis, although it failed to achieve regression in
fibrosis stage.61 Lanifibranor, a pan-peroxisome proliferatoractivated receptor agonist, achieved the endpoints of resolution of NASH and reversal of fibrosis compared with placebo
in phase II double-blind, randomized trial.62 Other potential
candidates for effective steatohepatitis treatment include
resmetirom, a selective thyroid hormone receptor-β agonist,63,64 and obeticholic acid, the selective farnesoid X receptor agonist.65-67 Of note, participants with CHB were excluded
from the above trials. Further investigations of these agents
aiming at the CHB subpopulation with concurrent steatohepatitis are urgently needed.
Another issue that should be noted is whether the correction of hepatic steatosis will cause an increase in HBV replicative activity. As mentioned previously, the inverse correlation
between hepatic steatosis and viral activity is evident, but
the exact mechanisms and the causal relationship are still
unknown, which means there is no clear recommendation
for CHB patients with hepatic steatosis undergoing correction of metabolic derangement. We recommend a short-interval monitoring plan which includes the blood test for ALT

https://doi.org/10.3350/cmh.2022.0422

http://www.e-cmh.org


levels (with or without HBV viral load) every three months during the correction. The optimal strategy warrants more clinical and mechanistic studies.

**Aggressive correction of metabolic dysfunction in CHB patients**

Factors of metabolic dysfunction like DM, obesity, or dyslipidemia are the essential components for MAFLD, and they are also well-established risk factors of fibrosis progression and HCC development among CHB patients. In a prospective study of 663 treatment-naïve CHB patients with serial liver stiffness measurements, metabolic syndrome, central obesity, and low level of high-density lipoprotein cholesterol were independently associated with liver fibrosis progression regardless of the change in viral load and ALT levels. The adverse influence was recently confirmed even in those receiving anti-viral treatment. In a large cohort study based on population-wide data from Taiwan and Hong Kong, the presence of DM was one of the reliable risk score variables to predict HCC occurrence in CHB patients receiving entecavir or tenofovir. In a prospective study of 5,754 CHB patients receiving NA in China, central obesity was associated with a two-fold risk of HCC before and after PSM. Among patients with confirmed MAFLD, the additive metabolic risk abnormalities, especially DM, are known to be associated with higher cardiovascular, cancer, and all-cause mortality. Similarly, in a recent Korean nationwide cohort study of 317,856 CHB patients, the metabolic risk factor burden increased the risks of HCC, non-HCC cancers, and all-cause mortality in a dose-dependent manner. Unlike steatosis, these metabolic risk factors seem to independently facilitate fibrosis and hepatocarcinogenesis without the interaction with HBV activity, so the aggressive correction of them is the key to better prognosis in both CHB and the general population regardless of the presence of steatosis.

**Unsolved questions**

A few issues must be addressed to optimize management in patients with concurrent CHB and MAFLD. First, considering the heterogeneous subpopulation within the MAFLD criteria, a better risk stratification strategy is required; those with different types of metabolic dysfunction may have distinct clinical characteristics and prognoses, and the impacts of these factors may be additive. For example, the presence of both DM and obesity should strengthen the indication for a more intensive follow-up schedule compared to those with only one or no metabolic risk factor. Second, since the concurrent steatosis leads to potential suppression of viral activi-
ty and resultant hepatocarcinogenesis in CHB, whether the simple steatosis alone (without other systemic risk factors of metabolic dysfunction) is tolerable or even favorable in the specific population such as CHB patients warrants more clinical studies to conclude. Third, how the therapeutic candidates for MAFLD, like GLP-1 agonist, influence the disease course and prognosis of CHB is still being determined due to the exclusion by trials and should be answered by the following real-world or post-marketing clinical trial data in the future.

CONCLUSIONS

Since the re-definition of MAFLD, in patients with CHB, several unsolved issues from mechanistic interaction to medical approaches warrant future investigations. Exploration of the mechanisms of the inverse correlation between steatosis and viral activity will help understand HBV virology which may be necessary for developing effective pharmacotherapy for HBV. Well-designed clinical trials focusing on optimal treatments for CHB patients with concurrent MAFLD are needed. As the increasing disease burden of metabolic syndrome worldwide, appropriate and timely action with multidisciplinary integration based on updated evidence will pave the way to the ultimate goal of enhancing prognosis and quality of life for the CHB population.

Authors’ contribution

Review design: Huang SC, Liu CJ. Analysis and interpretation of papers: Huang SC, Liu CJ. Drafting of the manuscript: Huang SC. Critical revision of the review: Liu CJ.

Acknowledgements

We acknowledge the support from the National Science and Technology Council (NSTC) and the Ministry of Health and Welfare (MOHW), Taiwan.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES


56. Allen AM, Hicks SB, Mara KC, Larson JJ, Therneau TM. The risk of incident extrahepatic cancers is higher in non-alcoholic fatty liver disease than obesity - A longitudinal cohort study. J Hepatol 2019;71:1229-1236.


What should be done to reduce the discrepancy between guidelines and real-life practice for hepatocellular carcinoma in Korea?

Min Kyung Park and Yoon Jun Kim
Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

Keywords: Hepatocellular carcinoma; Practice guideline; Surveillance; Diagnosis; Treatment

Hepatocellular carcinoma (HCC) is the sixth most common cancer with the third highest mortality rate worldwide.1 It is the seventh most common cancer in South Korea, and its crude incidence rate has not decreased over the past decade, resulting in a high socio-economic burden.2 Therefore, the Korea Liver Cancer Association (KLCA) and the National Cancer Center (NCC) of Korea collaborated to create the first HCC guidelines in 2003, which have been revised five times to date.3-5 The latest edition of the KLCA-NCC Korea practice guidelines were recently announced in 2022.6 The 2022 guidelines are evidence-based guidelines that analyze and systematically review the latest international research findings. In particular, the guidelines explore the systemic treatment of HCC, which has been developing immensely in recent years. Therefore, first-line treatments for HCC were newly established according to the trend of international guidelines. However, there are still several gaps between real-world practices and guideline recommendations. Accordingly, in this Clinical and Molecular Hepatology issue, Goh et al.7 concisely reviewed the 2022 KLCA-NCC guidelines and added real-life situations and practices.

For the surveillance of HCC, the 2022 KLCA-NCC guidelines recommend ultrasound and the serum alpha-fetoprotein for high-risk groups (i.e., patients with chronic hepatitis B, chronic hepatitis C, and liver cirrhosis). Since 2003, the Korean government has provided HCC surveillance for high-risk groups through the National Liver Cancer Screening Program (NLCSP), and the effectiveness of this surveillance program has been proven through previous studies.8,9 However, additional strategies are required since approximately 40% of all HCCs are still detected at an advanced stage.10 Evidently, many studies have been conducted to increase the effectiveness of surveillance through other imaging modalities, such as dynamic contrast-enhanced computed tomography or magnetic resonance imaging (MRI), or biomarkers.11 However, issues regarding radiation hazards and cost-effectiveness still persist. Therefore, guidelines have no choice but to use an ambiguous expression recommending alternative imaging modalities when ultrasounds are ineffective. In real-world
clinical practice, alternative imaging is widely performed based on the clinician’s judgment, and additional research is needed to identify specific individuals that require alternative imaging. In addition, the fact that only 52.7% of high-risk individuals participate in the NLCSF remains an obstacle to the early detection of HCC. Therefore, it seems necessary to classify and educate HCC high-risk individuals in public policy.

Regarding HCC diagnosis, the 2018 KLCA-NCC guidelines have allowed the diagnosis of HCC to be undertaken through liver MRI using a hepatobiliary agent to detect the washout appearance in the hepatobiliary phase, which confirms the diagnosis. Compared with the 2018 Liver Imaging-Reporting and Data System guidelines, one of the widely recognized clinical guidelines for HCC internationally, the 2018 KLCA-NCC guidelines, showed better sensitivity than hepatobiliary agent-MRI without compromising specificity. Therefore, the 2022 KLCA-NCC guidelines maintain the use of liver MRI for diagnosing HCC and are of great help in actual clinical practice.

The KLCA-NCC guidelines suggest the modified Union for International Cancer Control (mUICC) staging system as the primary staging system for HCC, and that treatment should be decided in accordance with mUICC staging. However, the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver use the Barcelona Clinic Liver Cancer (BCLC) staging system, which reflects liver dysfunction. Therefore, limitations in both research and interaction with the international community are unavoidable. Moreover, since BCLC staging is widely used in clinical settings, adding BCLC staging as a complementary system could be considered in future guidelines.

The 2022 KLCA-NCC guidelines for treating HCC provide the best and alternative options according to the mUICC staging system. Moreover, the systemic treatment part, which has been rapidly developing recently, has been updated according to the latest trends. According to the results of the IMbrave150 study, the drug combination of atezolizumab plus bevacizumab was suggested as a recommended first-line treatment option. For patients who are not indicated for such immune-modulating drug combination therapies, e.g., patients with autoimmune diseases, patients taking immunosuppressive drugs, patients who have undergone stem cell or solid organ transplantations in the past, and patients with high-risk bleeding tendencies, etc., either sorafenib or lenvatinib is recommended. For patients who have already undergone first-line treatment, the 2022 KLCA-NCC guidelines suggest various second-line treatment options at the level of expert opinion. However, insurance in Korea cannot cover such treatment options, so there is confusion in selecting second-line treatment options following these guidelines. Furthermore, in current clinical practice, various treatment options are used, taking advantage of the experience and conditions of each medical center, despite the guideline recommendation of the best options. Conversely, some treatment options, such as living donor liver transplantation or transarterial radioembolization, can only be applied to select patients due to limited resources or high costs.

The 2022 KLCA-NCC guidelines concisely describe the up-to-date findings through a systematic review, but inconsistencies with reality may occur in several aspects. Goh et al. described that the cause of this discrepancy may be confusion due to the absence of high-quality studies, and that some diagnostic tools and treatment methods are not strongly recommended due to high costs or limited resources. Furthermore, South Korea’s national reimbursement system does not guarantee that all examination and treatment options for HCC given in the KLCA-NCC guidelines will be provided, which strongly impacts actual medical practice. Furthermore, it is thought that differences arising in the resources or experience of medical staff within each medical institution may cause discrepancies between clinical practice and guidelines.

Diverse efforts are needed to minimize discrepancies between the guidelines and real-world practices. First, it is necessary to accumulate high-quality evidence-based research to answer critical questions. For example, to recommend alternative imaging modalities or biomarkers as guidelines, reliable domestic research on their cost-effectiveness is re-

---

**Abbreviations:**
BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; LXCA, Korea Liver Cancer Association; MRI, magnetic resonance imaging; mUICC, modified Union for International Cancer Control; NCC, National Cancer Center; NLCSF, National Liver Cancer Screening Program
quired. Large-scale studies are also necessary to determine the specific groups of patients that require alternative imaging modalities. Additionally, various systemic treatments for HCC have been developed, and the recommended first-line treatments have been established in large randomized controlled trials. However, there is still a lack of comparative studies on second-line treatments, making it challenging to recommend specific drugs in guidelines. Therefore, additional research is necessary to compare different second-line treatment options and to determine the most effective treatment for patients with specific characteristics. Second, South Korea’s insurance system should be changed to reflect the rapidly evolving medical environment and physicians’ consensuses. Third, it is necessary to acquire the latest updated knowledge and to consider the best treatment that can be provided from available resources within the medical society. Finally, patients should do their best to understand and actively participate in the prevention, monitoring, diagnosis, and treatment of HCC.

Authors’ contribution
Study conceptualization: YJK; Drafting of the manuscript: MKP; Critical revision of the manuscript: YJK.

Conflicts of Interest
Dr. Kim YJ reports receiving research grants from BTG, Boston Scientific, AstraZeneca, Gilead Sciences, Samjin, BL&H, and Bayer, and lecture fees from Roche, Abbvie, Eisai, Boston Scientific, BMS, BTG, Bayer, MSD, Gilead, Novo Nordisk, Green Cross Cell, Boehringer Ingelheim, and Gilead.

REFERENCES
Toward user-friendly and evidence-based practice guidelines for hepatocellular carcinoma

Do Young Kim
Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea

Keywords: Evidence; Guidelines; Hepatocellular carcinoma

Among the regional or national practice guidelines for hepatocellular carcinoma (HCC), the Korean Liver Cancer Association (KLCA)-National Cancer Center (NCC) guidelines had unique characteristics, particularly in assigning treatment modalities in each stage. Unlike the American Association for the Study of Liver Diseases (AASLD) or European Association for the Study of Liver diseases (EASL), KLCA-NCC guidelines adopted a modified Union for International Cancer Control (mUICC) staging system since the initial version in 2003. Adopting mUICC rather than the Barcelona Clinic Liver Cancer (BCLC) staging system has an advantage of encompassing heterogeneous tumor statuses and allocating the best or alternative treatment options to specific status, although it has a disadvantage of difficulty in international communication.

Adhering strictly to medical findings can hinder clinicians from applying guidelines to real practice because there are many differences between ‘the ideal’ and ‘the real’. On the contrary, consensus or expert opinion-dominated guidelines with a weak scientific background will be rejected by both physicians and government policy makers. The KLCA-NCC guidelines were assessed along with other 22 other regional or national guidelines regarding the overall quality and several domains of appraisal including scientific rigor and clarity of presentation. In the overall evaluation, the KLCA-NCC guidelines ranked third and were recommended for use without any modification.

In this issue, Goh et al. select key recommendations in surveillance, diagnosis, staging, and treatment and focus on the gaps between the revised KLCA-NCC guidelines and real practice. In surveillance, there is no difference of recommendation between 2022 and 2018 versions. Patients with cirrhosis (evidence level A, recommendation level 1), chronic hepatitis C (B1), and chronic hepatitis B (A1) are recommended to receive semiannual tests of serum alpha-fetoprotein (AFP) and ultrasonography (US). For non-cirrhotic patients with hepatitis C who achieved sustained virologic response following antiviral treatment, it is unclear whether those with low FibroScan score require continued HCC surveillance. Another difference between guidelines and real life is that contrast-enhanced computed tomography (CT) or magnetic res-
onance imaging (MRI) shows higher performance in HCC detection than US.\textsuperscript{1,4} Due to the limitations of contrast agent use, radiation, and high cost, there is little possibility that imaging methods using contrast agents will be alternative surveillance tools. On the other hand, accumulating data suggest that non-contrast MRI also has higher performance than US.\textsuperscript{1,5,6} Reflecting this, updated guidelines in 2022 included unenhanced MRI as a potential surveillance method with low evidence and recommendation level.\textsuperscript{7} Noninvasive diagnostic criteria for HCC changed from the 2014 to 2022 guidelines due to the introduction and increased use of liver-specific MRI contrast. In the 2014 version, even liver nodules smaller than 1 cm could be diagnosed as HCC based on contrast-enhanced CT or MRI using liver-specific contrast.\textsuperscript{8} However, 2018 and 2022 guidelines removed the diagnostic criteria for HCC in a liver nodule smaller than 1 cm because diagnostic performance of imaging modalities was low in histologically confirmed subcentimeter-sized HCC.\textsuperscript{9} This leaves uncertainty for contrast-enhanced CT or MRI showing compatibility with subcentimeter HCC. It is important to remember that guidelines cannot include all clinical situations, and it is ultimately up to the physician whether to observe or treat the lesion. If the lesion is new and located near a vessel and the AFP level is greater than 100 ng/mL, treatment rather than observation might be rational. In the 2022 guidelines, ancillary imaging features provide additional information that may change a diagnosis from definite to probable HCC. That is, HCC diagnosis cannot be concluded without radiologic hallmarks of arterial hyperenhancement with washout in the portal, delayed, or hepatobiliary phase. The guidelines suggest repeat imaging within 3–6 months or biopsy for probable HCC diagnosed based on ancillary features. It is unknown whether physicians in real practice will follow this recommendation.

As aforementioned, the main strength of KLCA-NCC guidelines is the basis of the mUICC staging system, enabling application to heterogeneous tumors from stage I to IVb. For each stage, the best and alternative options are presented to enhance practical applicability. In particular, the 2022 version added quality of evidence as a factor of the best option, allowing physicians to make decisions based more strongly on medical findings. While BCLC staging strictly assigns surgical resection to very early or early stage disease with preserved liver function and without portal hypertension, most surgeons from both Eastern and Western countries insist that indication of surgical resection should be expanded to multiple HCCs and HCC with limited vascular invasion (Vp 1-2).\textsuperscript{11,12} Considering this, the 2022 guidelines maintain the recommendation of surgery for HCC with limited vascular invasion and added the recommendation of resection for multiple HCCs with low evidence and recommendation levels. In real practice, selection of surgical resection in this population is not common. However, with the recent success of adjuvant immune checkpoint inhibitor treatment, resection of HCC with high risk will be performed more frequently.\textsuperscript{13} Regarding the role of transarterial radioembolization (TARE), although guidelines suggest that it can be applied widely from stages I to III, resection remains the first recommendation in the early stage (I), followed by conventional transarterial chemoembolization (TACE) in the intermediate stage (II) and to systemic treatment in the advanced stage (III). Owing to the failure of two randomized trials comparing TARE and sorafenib in unresectable, advanced HCC,\textsuperscript{14,15} the initial strategy of loco-regional treatment using Yttrium-90 microspheres seems to be chosen at earlier stages. Although a recent study suggested that combination treatment with TARE and immune checkpoint inhibitor treatment would increase the therapeutic efficacy in advanced HCC, more evidence is necessary for this kind of treatment to be included in the guidelines. As in other guidelines, the updated 2022 KLCA-NCC guidelines recommended atezolizumab+bevacizumab or durvalumab+tremelimumab for first-line systemic treatment based on phase III trials.\textsuperscript{16,17} The optimal second-line systemic therapy remains to be determined. As all well-designed clinical trials on second-line treatment followed first-line sorafenib, data are lacking on second-line therapy following atezolizumab or atezolizumab+lenvatinib.\textsuperscript{18} The guidelines committee held a ‘delphi’ meeting to achieve a consensus on the available second-line systemic therapies following tyrosine kinase inhibi-
tor or an immune agent. As a result, evidence level ‘D’ involving expert opinion was referenced in the recommendation of second-line systemic therapy.

As the authors stated, there are differences between recommendations based on guidelines and real world practice. These differences are due to lack of evidence or a reimbursement system, limiting the use of certain drugs based on approval issues. For a guideline to be assessed qualified, it should be based on evidence, user-friendly and fully consider real daily practice. In this regard, the updated KLCA-NCC guidelines are based on both clear scientific evidence and expert opinion, producing a more use-friendly guide.

Conflicts of Interest

The author has no conflicts to disclose.

REFERENCES

The clinical management of hepatocellular carcinoma in China: Progress and challenges

Shan Shan and Jidong Jia
Liver Research Center, Beijing Friendship Hospital, Capital Medical University; The National Clinical Research Center for Digestive Diseases, Beijing, China

Keywords: Hepatocellular carcinoma; Guideline; Treatment

Primary liver cancer, which mainly comprises hepatocellular carcinoma (HCC), poses a significant public health burden, especially in China and other Asian countries/territories.1,2 The major etiology of HCC in China is chronic hepatitis B virus (HBV) infection, which confers a more malignant phenotype in terms of higher serum level of alpha-fetoprotein (AFP) and rapid progression.3,4 Primary prevention through universal vaccination against hepatitis B has successfully reduced HCC incidence and mortality in China.5 In prospective randomized clinical trials, screening and surveillance of HCC in high-risk populations identified more cases at early stages and improved the clinical outcomes compared with no surveillance.6,7 High-quality research from China on the diagnosis, staging, and treatment of HCC has provided important evidence for developing guidelines.8-10

In an article published in the current issue of *Clinical and Molecular Hepatology*, Dr. Xie and colleagues11 from Zhongshan Hospital, Fudan University, Shanghai, China, provided an excellent overview of the advances in the clinical management of HCC in China. This review article depicted the key points of the 2022 updated guidelines and the big picture of real-world clinical HCC management in China, while focusing on the rationale and evidence supporting the recommendations. First, the surveillance of HCC by AFP measurement and ultrasonography every six months is recommended in the high-risk population, and the diagnosis of suspected nodules can be confirmed by enhanced multiphasic CT/MRI. Second, the China Liver Cancer Staging System (CNLC) is recommended for use because it is better reflective of individual subgroup survival, facilitating allocation of therapeutic modalities. Third, the University of California San Francisco criteria for liver transplant are adopted and offer HCC patients more transplant opportunities than the Milan criteria but yield a similar post-transplant outcome. Last, this article also discussed multimodal and high-intensity anti-tumor strategies for HCC patients in real-life practices in China, such as the addition of immunotherapy-based systemic therapy to local modalities, which may improve the chance of receiving cura-
tive therapy and long-term survival for patients with non-
early-stage HCC.

Although great progress has been achieved, control and management of HCC remain significant challenges in China. First, the total burden of HCC is high, although the age-standardized incidence and mortality rates are declining. The high burden is mainly due to the huge number of people living with chronic HBV infection, who carry a high risk of HCC development and require long-term antiviral therapy to reduce progression to cirrhosis and HCC. However, the diagnosis and treatment rates of chronic HBV infection are low despite the increasing trend. Second, due to poor adherence to long-term HCC surveillance, most patients with HCC are in intermediate or advanced stage, which carries a very poor survival rate since such patients are not eligible for curative therapy. Third, compliance with clinical HCC management guidelines must be improved. Real-life practice of HCC management is highly heterogeneous and subject to personal opinion, local expertise, or available resources rather than clinical study evidence and guideline recommendations. Last, survival disparity exists between populations with different insurance types, which may reflect the different socioeconomic statuses of the patients.

To meet the aforementioned challenges, consensus on the primary, secondary, and tertiary prevention methods of primary liver cancer has been published in China in 2018, 2021, and 2022, respectively. Universal infant HBV vaccination combined with a triple elimination program to prevent mother-to-child transmission of human immunodeficiency virus, syphilis, and HBV, together with large-scale diagnosis and treatment of CHB will eventually reduce the incidence of HCC. Population-based cancer screening and surveillance programs, including those for HCC, in rural areas are well-planned and pending implementation. HCC surveillance will increase the opportunity for curative treatment. Furthermore, the advent and validation of novel serum biomarkers and models may also facilitate surveillance and identification of early-stage HCC. Finally, the timely updating and advocating of evidence-based HCC guidelines will help improve the quality of care in real-world practice to improve clinical outcomes of HCC patients in China.

Authors’ contribution
Drafting of the manuscript: Shan Shan; Critical revision of the manuscript: Jidong Jia.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 8200569 and 82270603).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Management of hepatocellular carcinoma in China: Seeking common grounds while reserving differences

Tian Yang, Ming-Da Wang, Xin-Fei Xu, Chao Li, Han Wu, and Feng Shen
Department of Hepatobiliary Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University (Naval Medical University), Shanghai, China

Keywords: Hepatocellular carcinoma; China; Hepatectomy; Molecular targeted therapy; Immunotherapy

Hepatocellular carcinoma (HCC) is a serious and life-threatening form of cancer that is highly prevalent in China due to the high prevalence of hepatitis B virus (HBV) infection. Other risk factors for HCC include hepatitis C virus (HCV) infection, exposure to aflatoxins, excessive alcohol consumption, and tobacco smoking. In China, neonatal HBV vaccination programs and effective anti-viral agents have contributed to a significant decline in HCC incidence, especially for those below 40 years old. However, the increasing prevalence of HCV, diabetes mellitus, obesity, non-alcoholic fatty liver disease (NAFLD), and other risk factors for HCC is concerning and could lead to an increase in the number of cases of HCC in the future.

Prevention and early detection of HCC are important in order to reduce the burden of this disease. The aMAP score (prognostic score involving age, male, albumin-bilirubin and platelets) is recommended in the 2022 Chinese guidelines to predict HCC occurrence, and the China liver cancer (CNLC) staging system proposed in the 2017 guideline is the standard model for staging. Multi-modal and high-intensity strategies treatments, including the addition of immunotherapy-based systemic treatment to local therapies such as liver resection, local ablation, and intra-arterial therapies, have been adopted in real-life practices in China. The treatment options for HCC vary depending on the stage of the disease. According to the Bridge to Better Outcomes in HCC (BRIDGE) study, unfortunately, only 36% of Chinese cases were initially diagnosed at an early stage and eligible for curative treatments, including liver resection, local ablation and liver transplantation, while the remaining 9% and 55% were at intermediate and advanced stages, respectively.

HCC surveillance is an important tool for early detection
and improved long-term survival, yet adherence to surveillance protocols is low even in high-risk patient populations. This is due to a variety of factors, including poor access to medical care, higher prevalence of comorbidities, etiology of liver disease, and less favorable socioeconomic status. It is essential that physicians assess patients holistically when making decisions on the appropriateness and necessity for HCC surveillance, taking into account all known risk factors, including demographics, co-morbidities, environmental factors, fibrosis stage, medications, serologic tests and genetic polymorphisms. 

Multidisciplinary treatment (MDT) is essential for the successful management of HCC, as it allows for the integration of different medical specialties to provide comprehensive care for patients. MDT is beneficial in providing a holistic approach to the diagnosis and treatment of HCC, and it is also important in helping to identify the most appropriate treatment option for each individual patient. However, due to too many patients with newly diagnosed HCC every year, it is difficult to provide MDT for every patient with HCC, especially in China. Due to the involvement of multiple departments in the MDT of HCC, including hepatobiliary surgery, liver transplantation, interventional therapy, radiation therapy, digestive medicine, oncology, infectious diseases, minimally invasive ablation, and even traditional Chinese medicine, there is currently a situation of competition among departments for HCC patients in many Chinese hospitals. As we believe, similar situations also exist to a greater or lesser extent in other countries and regions worldwide. The availability of low-cost treatments for HCC in China is encouraging, but the high prevalence of advanced and end-stage disease in rural areas means that many patients are unable to access these treatments in time. Furthermore, the long-term, continuous, and comprehensive nature of HCC treatment means that some patients are unable to continue treatment due to the cost. It is therefore important to ensure that all patients have access to affordable and effective treatments for HCC.

The CNLC system incorporates tumor characteristics, liver function and performance status, similar to the Barcelona Clinic of Liver Cancer (BCLC) system. Each stage of BCLC 0/A, B, and C is divided into two substages in the CNLC system, including stages Ia, Ib, Ia, IIb, IIa and IIb, with CNLC stage IV equivalent to BCLC stage D. The CNLC system is a treatment allocation method for decision-making purpose, while the Japan Integrated Staging (JIS) score and its variants focus on the prognostic predictive function. The modified Union for International Cancer Control (mUICC) system adopted in Korea is characterized by more detailed treatment allocation and is applied on the premise of Child-Pugh A function, no portal hypertension and performance status (PS) scoring 0–1. The CNLC system has been widely used in real-life practices in China, although the BCLC systems continues to be the main stratification factor for clinical trial designing.

With regard to the patterns of hepatic resection for HCC, including patient characteristics, candidate selection, operative techniques, and surgical practice, etc., distinct differences between those hepatic surgical centers in the East and the West have been widely acknowledged. However, those studies providing meaningful direct comparisons have been lacking, especially for their surgical safety and long-term efficacy – that is, which one side is superior to the other side is little based on the evidence till now. In our previous study, there were many significant differences in patient characteristics and operative variables for their patients undergoing liver resection for HCC between two large hepatic surgical centers in the East (Eastern Hepatobiliary Surgery Hospital of Shanghai) and the West (Mount Sinai Hospital of New York), but no any difference was revealed in their hospital mortality and morbidity rates (both overall and major), as well as their long-term survival and recurrence. In other words, the present study demonstrates that the safety and efficacy of liver resection for HCC are comparable between these two centers which could be regarded as representative ones from the East and the West.

BCLC intermediate and advanced stage (BCLC stage B and C) HCC is a heterogeneous population, and that PS and tumor features should be taken into account when determining the best treatment for each patient. PS1 alone is not sufficient to include a patient into either of these two stages, and that new patient-tailored therapeutic indications are needed in order to improve overall survival rates.

---

**Abbreviations:**

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; CNLC, China liver cancer; MDT, multidisciplinary treatment; BCLC, Barcelona Clinic of Liver Cancer; JIS, Japan Integrated Staging; mUICC, modified Union for International Cancer Control
China is making significant efforts to tackle HCC, with the Ministry of Health investing heavily in viral hepatitis and HCC research and the Chinese Academy of Sciences setting up a national tumor research center in Shanghai. These initiatives have already led to an increase in the number of Chinese research papers on HCC, but more international cooperation is needed to further upgrade China’s scientific research in HCC and improve prevention and treatment of the disease. For all clinicians of the world, whether from China or elsewhere, "seeking common ground while reserving differences", this is the core of objective and unbiased academic attitude we need to set up when facing the issue of HCC management.

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

Acknowledgements
This study was supported by the National Natural Science Foundation of China (No. 81972726 and 82273074 for Yang T), Dawn Project Foundation of Shanghai (No. 21SG36 for Yang T), and Shanghai Health Academic Leader Program (No. 2022XD001 for Yang T).

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Editorial

The prime time for management of hepatocellular carcinoma in Hong Kong

Landon L. Chan¹ and Stephen L. Chan¹,²

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

Keywords: Hepatocellular carcinoma; Immune checkpoint inhibitors; Chemoembolization; Lenvatinib

In this issue of Clinical and Molecular Hepatology, the article ‘the Hong Kong perspective of clinical management of hepatocellular carcinoma (HCC). In the past decade, we have witnessed remarkable advances on the surveillance, diagnosis, and treatment of HCC, which lead to changes in clinical practice and result in improved outcomes of patients. Due to the high disease burden and the mature healthcare system, Hong Kong is one of the earliest places in the world to adopt multidisciplinary care for HCC and to initiate research on HCC.

As highlighted in the review paper, the management of HCC has undergone a rapid development, and this is leading to another wave of practice-changing studies. For surveillance of HCC, the use of alpha-fetoprotein (AFP) and abdominal ultrasound (US) is well-known to be associated with improved early detection, curative treatment receipt and survival in patients with cirrhosis. However, as compared to other screening strategies, such as breast cancer with mammogram with a sensitivity of 77 to 95%, colorectal cancer with faecal immunochemical test with sensitivity of 79%, nasopharyngeal cancer with EBV-DNA with sensitivity of 97.1%, the sensitivity of combined AFP and US of approximately 60% for HCC is relatively lower. Recently, circulating cell-free DNA has gained popularity as a screening test for early detection of multiple cancers with encouraging results. In a phase II case-control study involving 401 patients, a multi-target HCC blood test panel using three methylated markers, in combination with AFP and sex, showed a sensitivity of 82% for early-stage HCC detection with a specificity of 87%. In addition, the Circulating Cell-free Genome Atlas study has recently reported the performance of a plasma cell-free DNA screening test using a panel of >100,000 informative methylation regions, with an overall sensitivity of 67.3% in a pre-specified set of 12 cancer types, including liver cancer. It is anticipated that cell-free DNA will be an important tool for surveillance in the near future.

Hui et al. depicted the real-life practice in diagnosis of HCC in Hong Kong, which is mainly based on non-invasive tests
with contrast-enhanced imaging to identify the characteristic features of HCC. This is largely compatible with the recommendation of international guidelines. However, there has recently been a swing back to obtain tissue diagnosis during work-up for HCC for two reasons. First, there is a growing recognition of alternative entities of liver malignancies including the intrahepatic cholangiocarcinoma (CC) and combined HCC-CC, which frequently require pathological diagnosis for confirmation. In fact, a recent large retrospective analysis in UK has shown that around 10% of advanced-stage liver disease would receive an incorrect diagnosis based on non-invasive radiological criteria. Second, given the increasing number of targeted therapy and immunotherapy for HCC, molecular subtyping of HCC is important to develop predictive biomarkers and the pursual of personalized treatment for HCC.

Following diagnosis of HCC, treatment of curative intent could be achieved by liver resection, ablation, or transplantation for early-stage disease. However, as noted by Hui et al., recurrence rates occurred in over 70% for patients following hepatectomy. Conventionally, effective adjuvant treatment has been lacking for HCC. This deadlock is expected to be broken in 2023 by the IMbrave050 study, which is a clinical trial randomizing 662 patients with HCC who had undergone curative resection or ablation, to receive adjuvant atezolizumab plus bevacizumab for up to 12 months, or no intervention. According to a recently announced press release, the prespecified interim analysis showed that patients in the experimental arm had statistically significant improvement in recurrence-free survival (RFS). The results are expected to be presented in major conferences in early half of 2023.

For intermediate-stage disease, transarterial chemoembolization (TACE) remains a standard treatment. However, TACE as the only recommended treatment in this heterogeneous group has recently been challenged. On one hand, systemic therapy is increasingly considered a better alternative for selected high-burden intermediate-stage HCC. In fact, in the latest 2022 updated version of treatment recommendation based on BCLC staging, TACE is no longer recommended as treatment for intermediate-stage disease when patients have high-burden HCC (e.g., extensive bilobar liver involvement, diffuse, infiltrative HCC). In a proof-of-concept retrospective propensity score-matched study, the use of lenvatinib in intermediate-stage “up-to-7” out HCC was associated with improved OS (37.9 months vs. 21.3 months, \( P < 0.01 \)), progression-free survival (PFS) (16.0 months vs. 3.0 months, \( P < 0.001 \)) and objective response rate (ORR) (73.3% vs. 33.3%, \( P < 0.001 \)) as compared to TACE. \(^1\) The study also showed that hepatic function deteriorated with repeated TACE (baseline ALBI score from -2.66 to -2.09, \( P < 0.001 \)) but was maintained in the group treated with lenvatinib (baseline ALBI score from -2.61 to -2.61, \( P = 0.254 \)). The use of more aggressive systemic therapy with atezolizumab plus bevacizumab in the intermediate-stage HCC is currently underway by ongoing randomized study (NCT04803994). On the other hand, the addition of systemic therapy to TACE could improve the outcome of intermediate-stage HCC. According to the TACTICS-L study, a phase II single-arm study, the combination of lenvatinib with TACE in intermediate-stage unresectable HCC patients was associated with a ORR up to 88.7% with complete response seen in 66.1% of patients. The median PFS was 28.3 months, which had already approached the expected OS of intermediate-stage HCC based on available scientific evidence. The randomized study from China also suggested that the addition of lenvatinib to TACE could improve both ORR, PFS and OS of both intermediate- and advanced-stage HCC. The above data unanimously suggest that systemic therapy has an important role in intermediate-stage HCC.

As mentioned by Hui et al., immunotherapy or immunotherapy containing regimes are increasingly advocated as their efficacy has been demonstrated in a number of landmark trials such as IMbrave150, CheckMate040 and HIMALAYA. As a result, immunotherapy is recommended in both the first-line and subsequent-line settings according to local and international guidelines. For all phase III clinical trials testing immunotherapy in advanced-stage HCC, patients with Child-Pugh A liver function were mostly recruited. However, a significant proportion of patients with Child-Pugh B liver function with advanced disease are frequently seen in the clinic at presentation. Recently, data has emerged that single agent nivolumab for Child-Pugh B unresectable HCC could be safe and effective, and thus now being incorporated.

**Abbreviations:**

HCC, hepatocellular carcinoma; AFP, alpha-feto protein; US, ultrasound; CC, cholangiocarcinoma; RFS, recurrence-free survival; TACE, transarterial chemoembolization; PFS, progression-free survival; ORR, objective response rate

https://doi.org/10.3350/cmh.2023.0094
into the latest Hong Kong consensus statement.\(^1\) The CheckMate040 study (cohort 5), a phase I/II study testing single agent nivolumab in patients with advanced HCC and Child-Pugh B cirrhosis, was published.\(^24\) It demonstrated clinically meaningful stabilization of liver function, and improved OS compared to historical cohort treated with sorafenib (7.6 months vs. 2.5–5.4 months).\(^24\) Although in the CheckMate040 study, patients with Child-Pugh B7 liver function had similar OS compared to those with Child-Pugh B8 liver function, a couple of retrospective real-world studies have shown that patients with Child-Pugh B7 liver function derived more benefits from nivolumab than Child-Pugh B8.\(^{25,26}\) Therefore, nivolumab could represent a potential treatment option in advanced HCC with Child-Pugh B7 liver function if it is validated in a larger cohort of patients. Overall, “the Hong Kong perspective of clinical management of HCC” written by Hui et al.\(^1\) is a timely piece of summary of the key advancements and real-life practice of HCC management in recent years in Hong Kong. Emerging breakthroughs are budding in all fronts of HCC management, including surveillance, diagnosis, and management of different stages of disease. We eagerly await these exciting results to come, and it will be the prime time for HCC in the coming years.

**Authors’ contribution**

The authors contribute equally in the drafting and editing of the manuscript.

**Conflicts of Interest**

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

**REFERENCES**


Clinical practice guidelines and real-world practice for hepatocellular carcinoma in Taiwan: Bridging the gap

Shen-Yung Wang
Division of Gastroenterology and Hepatology, Department of Medicine, MacKay Memorial Hospital, Taipei, Taiwan

Keywords: Hepatocellular carcinoma; Surveillance; Systemic therapy

Hepatocellular carcinoma (HCC) is highly prevalent in Taiwan, with liver cancer being the second leading cause of cancer-related mortality and a major threat to public health. Many efforts including a nationwide vaccination program against hepatitis B virus, Taiwan Cancer Registry, and National Health Insurance (NHI) program have been developed to improve the prevention and management of HCC. Additionally, the Taiwan Liver Cancer Association (TLCA) and Gastroenterological Society of Taiwan (GEST) collaborated to develop a management consensus for HCC to provide guidance and advice to physicians. However, discrepancies exist between the updated evidence of care for HCC and real-world practice. Taiwan’s NHI program accounts for the majority of health care expenditures in the country, and its policy has significant impacts on real-world practice for HCC by influencing the availability and accessibility of diagnostic tools and treatments. Nevertheless, factors such as patient adherence, comorbidities, attitudes and awareness of physicians, and treatment availability also play an important role in real-world treatment of HCC.

In the current issue of Clinical and Molecular Hepatology, Su et al. reviewed the current status of HCC management in Taiwan and compared clinical guidelines with real-world practice for HCC treatment. Regular surveillance is crucial in HCC management as it improves the outcomes of patients by increasing the likelihood of receiving curative therapy. Risk prediction models for HCC stratify patients into risk groups for HCC, and surveillance in patients at high risk of HCC has been shown to improve prognosis. The TLCA guideline advocates regular screening for HCC especially in at risk patients; although this screening is reimbursed by the NHI, patient adherence to surveillance is poor. Integration of biomarkers such as serum alpha-fetoprotein (AFP) has been recommended in HCC surveillance in Taiwan. Protein induced by vitamin K absence or antagonist II (PIVKA-II; also known as des-γ-carboxylated prothrombin, DCP), a biomarker useful in the early diagnosis of HCC, has been recently reimbursed in Taiwan. HCC can be diagnosed with characteristic vascular
patterns in dynamic imaging methods including computed tomography (CT) or magnetic resonance imaging (MRI). Gadoxetic acid is a hepatocyte-specific MRI contrast agent, the combination of which (EOB-MRI) has been shown to be more accurate and sensitive than ultrasound (US) or CT in detecting small HCCs or differentiating HCC from benign focal liver lesions. EOB-MRI was recommended by the TLCA guidelines for early detection and assessment of staging and tumor burden of HCC. Contrast-enhanced US (CEUS) is superior to unenhanced US in the diagnosis of HCC, and CEUS with hepatocyte-specific contrast agents such as Sonazoid can provide Kupffer phase imaging for surveillance of HCC. The TLCA and other international clinical practice guidelines recommend the use of EOB-MRI and CEUS in the management of HCC, but neither is covered by the NHI in Taiwan.

Surgical resection remains the major treatment for early-stage HCC in Taiwan. Local ablation with radiofrequency or microwaves is recommended by TLCA guidelines and reimbursed as first-line treatment for early-stage HCC with tumor size less than 5 cm. Trans-arterial chemoembolization (TACE) is the first-line treatment for intermediate-stage HCC in Taiwan. Other treatments such as TACE with drug-eluting beads or radioembolization are not reimbursed. Systemic therapy for HCC has shown significant progress in recent years. Drugs including multi-tyrosine kinase inhibitors, anti-angiogenic agents, and immune checkpoint inhibitors (ICIs) have shown benefits for advanced HCC in randomized controlled trials. Taiwan guidelines recommend either sorafenib, lenvatinib, or a combination of atezolizumab with bevacizumab as first-line systemic therapy for advanced HCC. While other international clinical practice guidelines have recommended the combination of atezolizumab with bevacizumab as first-line therapy for advanced HCC, only sorafenib and lenvatinib are currently covered by NHI as first-line systemic therapy in Taiwan. Regorafenib or ramucirumab (for AFP ≥400 ng/ml) is reimbursed as a second-line therapy for HCC patients who progressed on sorafenib. None of the ICIs are currently covered by the NHI as either first- or second-line systemic therapy for HCC. Systemic therapy has shown to benefit intermediate-stage HCC and has been recommended in patients refractory or unsuitable for TACE. A wide range of heterogeneity in the combination of systemic agents has been shown in real-world practice in Taiwan. Analyzing the real-world practice data may be valuable for evaluation of treatment and outcomes of HCC, which may facilitate NHI approval and help the development of practice consensus.

HCC is a highly heterogeneous disease, and management requires a multidisciplinary approach based on the individual characteristics of patients including liver reserve, tumor burden, comorbidities, and underlying liver diseases. Bridging the gap between clinical guidelines and real-world practice for HCC also requires a multidimensional approach. Patient education and physician motivation should be strengthened to improve adherence to surveillance recommendations and early detection of HCC. Academic organizations may provide comprehensive guidance to integrate the use of biomarkers and imaging modalities to guide HCC treatment and may consider updating the recommendations according to recent advances in systemic therapy for HCC. The NHI coverage of diagnostic tools and treatments for HCC should consider embracing the most updated evidence to facilitate real-world practice and improve treatment outcomes for patients with HCC in Taiwan.

Conflicts of Interest
The author has no conflicts to disclose.

REFERENCES


Challenges in translating clinical guidelines into real-life practice for management of hepatocellular carcinoma in Taiwan

San-Chi Chen

Division of Medical Oncology, Department of Oncology, Taipei Veterans General Hospital; Faculty of Medicine, Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

Keywords: Hepatocellular carcinoma (HCC); Clinical practice guidelines; Taiwan; National health insurance

Hepatocellular carcinoma (HCC) is a prevalent and deadly disease that poses a significant public health challenge worldwide. Taiwan has one of the highest incidence rates of HCC in the world, and managing this disease has become a top priority for the country’s healthcare system. In recent years, clinical guidelines have been developed by the Taiwan Liver Cancer Association (TLCA) to provide evidence-based recommendations for surveillance, diagnosis, and treatment of HCC. However, implementation of these guidelines in real-life practice remains a challenge. In this editorial, we will discuss the paper by Su et al., who provide valuable insights into the challenges of translating clinical guidelines into real-life practice and highlight the need for further research to optimize HCC management in Taiwan.

First, surveillance and diagnosis will be discussed. Although the TLCA guideline recommends checking PIVKA-II every three months, insurance only reimburses this procedure twice a year since 2020. Regarding pathological diagnosis, although the probability of cancer cell dissemination caused by biopsy is very low, in early HCC cases that meet the diagnostic imaging criteria, surgery or radiofrequency ablation therapy is conducted directly. Some doctors may perform tissue biopsy followed by RFA. Tissue biopsy to confirm the diagnosis is recommended in the following situations: absence of risk factors of HCC, atypical imaging findings, suspicion of combined hepatocellular carcinoma-cholangiocarcinoma (HCC-CC), a low possibility of a primary liver tumor, low levels of AFP and PIVKA-II, and high CA 19-9/CEA levels. In suspected intermediate to advanced stage HCCs, a pathological diagnosis is required, and clinicians are increasingly performing biopsies on these patients. Differential diagnosis of HCC includes poorly differentiated tumor, sarcomatoid transformation, combined HCC-CC, or metastatic cancer. Due to the poor prognosis of these features, clinicians may adjust their treatment strategies. Therefore, more clinicians are performing tissue biopsies on newly diagnosed HCC patients as soon as possible. As Su et al. mentioned in their 2019 article,
up to 48.2% of HCC patients in Taiwan have received a histological diagnosis.

In terms of staging, although magnetic resonance imaging (MRI) or Gadolinium Ethoxybenzyl Diethylenetriamine Pentacetic Acid enhanced magnetic resonance imaging (Gd-EOB-DTPA-MRI) has higher sensitivity, CT scans remain the mainstay for staging in most liver cancer patients. In certain cases, surgeons may repeat MRI before surgery, such as when there are multiple tumors, infiltrating tumors, uncertainty about vascular invasion, or atypical enhanced images and suspected tumor rupture. Chest CT scans might not be performed routinely for very early stage HCC, although it is recommended that most patients undergo such scans to accurately define their stage and receive the most appropriate treatment.

Due to the scarcity of donation sources, liver transplantation is usually reserved for patients with poor liver function. Although HCC patients in Taiwan with good liver function meet the criteria for liver transplantation, they usually undergo local treatments such as surgery and embolization. Patients are only referred for liver transplantation if they experience repeated relapses after local treatments, and these patients usually receive a living-donor liver transplant.

In intermediate stage HCC, transarterial chemoembolization (TACE) is the most common local treatment. Drug-eluting bead-TACE (DEB-TACE) is not reimbursed by the National Health Insurance, and its timing of use is usually recommended by the interventional radiologist in a multidisciplinary team meeting. In Taiwan, patients who experience recurrence after three TACE trials within one year are considered refractory to the treatment. These patients can be reimbursed for target therapy, and clinicians often use target therapy alone or in combination with TACE to treat TACE-refractory HCC. Radio-embolization is relatively expensive, and only a small number of patients receive this treatment. In recent years, more hospitals in Taiwan have been able to perform radio-embolization, although more hospitals are also offering proton therapy as an option. Due to the limitation of the maximum radiation dose, patients can only choose one of these two treatment modalities. Hepatic arterial infusion chemotherapy (HAIC) is chosen by only a few doctors due to an insufficient level of evidence and the need for assistance from interventional radiologists. Currently, there is no consensus on the optimal timing of systemic therapy for intermediate stage HCC. Some physicians initiate systemic therapy in TACE-refractory HCC. In recent years, thresholds of tumor burden score up to 7 or 11 have been proposed. Patients with a smaller tumor burden in intermediate stage HCC usually undergo treatment with TACE. For cases with high tumor burden, the choice of TACE, systemic therapy, or combination therapy depends on the physician.

For advanced stage HCC, atezolizumab plus bevacizumab (atezo+bev) is the first-line treatment of choice. However, since insurance does not currently cover this combination, only about one-tenth of patients can afford the treatment. For patients who cannot afford atezo+bev treatment, target therapy is covered by insurance.

Currently, Taiwan’s National Health Insurance covers drugs such as sorafenib, lenvatinib, regorafenib, and ramucirumab (when AFP > 400 ng/mL). Approximately half of clinical physicians use sorafenib as the first-line treatment, as regorafenib or ramucirumab is covered by insurance only after sorafenib failure. Due to the high response rate and longer progression-free survival (PFS) of lenvatinib, it is used as first-line treatment by some clinicians, even though insurance does not cover other drugs after lenvatinib failure. Regarding PD-1 blockade, the National Health Insurance in Taiwan only provided reimbursement for second-line treatment with nivolumab to patients who failed sorafenib between 01/04/2019 and 31/03/2020. The efficacy of nivolumab was analyzed in 408 patients, and the ORR of 25% and the PFS of 2.9 months were similar to the results from clinical trials. Unfortunately, the efficacy of nivolumab was lower than that in other cancer types treated with PD-1 blockade covered by national insurance. Consequently, reimbursement of nivolumab was terminated.

Although clinical trials combining PD-1 blockade with multiple kinase inhibitor (MKI) have not been successful, physicians in Taiwan sometimes use such combination therapy. Three retrospective studies have provided real-world evidence for this approach. Our team used propensity score-matching to compare the efficacy of anti-PD-1 combined with sorafenib versus anti-PD-1 alone. The results showed

**Abbreviations:**
HCC, hepatocellular carcinoma; TLCA, Taiwan Liver Cancer Association; TACE, transarterial chemoembolization; DEB-TACE, drug-eluting bead-TACE; HAIC, hepatic arterial infusion chemotherapy; PFS, progression-free survival; MKI, multiple kinase inhibitor; OS, overall survival

that anti-PD-1 plus sorafenib had higher disease-control rate and longer PFS and overall survival (OS) than anti-PD-1 alone. In addition, we published another study showing that the combination of lenvatinib and nivolumab had better efficacy than lenvatinib alone. Furthermore, Wu et al. reported that the combination of lenvatinib and pembrolizumab had a high disease control rate and did not affect the ALBI (Albumin-Bilirubin) score. Unfortunately, the combination of lenvatinib and pembrolizumab was found to have limited benefit in the Leap-002 trial. Overall, these off-label combination therapies require a high-level of evidence to confirm their benefits.

In summary, clinicians in Taiwan are highly active in treating HCC and are hoping for early approval of immunotherapy reimbursement from the National Health Insurance. The clinical experience gained in Taiwan can serve as a useful reference for scholars globally involved in treatment of HCC.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

The latest global burden of liver cancer: A past and present threat

Joo Hyun Oh¹ and Dae Won Jun²

¹Department of Medicine, Nowon Eulji Medical Center, Eulji University School of Medicine; ²Department of Medicine, Hanyang University College of Medicine, Seoul, Korea

Keywords: Global burden of disease; Epidemiology; Liver neoplasms

Liver cancer is the fourth most common cause of death globally, accounting for over 800,000 deaths annually.¹,² Hepatocellular carcinoma represents approximately 90% of primary liver cancers, followed by intrahepatic cholangiocarcinoma and other primary liver malignancies. Approximately 90% of hepatocellular carcinomas are associated with a known underlying cause, most commonly chronic viral hepatitis, heavy alcohol use, and non-alcoholic fatty liver disease.³ The distribution of these etiologies differs geographically.⁴ For instance, East Asia has a higher prevalence of chronic viral hepatitis, while heavy alcohol use is highest in Europe. The incidence and mortality rates of liver cancer also differ regionally.⁵

In this issue of Clinical and Molecular Hepatology, Choi et al.⁶ analyzed data from the Global Burden of Disease 2019 on primary liver cancer. A Global Burden of Disease report has shown recent trends in liver cancer. Between 1990 and 2019, the crude number of disease-adjusted life years (DALYs) and deaths in 204 countries increased from 11,278,630 to 12,528,422 and from 365,215 to 484,577, respectively. A couple of years ago, Akinyemiju et al.⁷ reported on the global burden of liver cancer by 2015 using Global Burden of Disease data. The number of incident liver cancer cases in 2015 was 854,000, whereas the number of deaths and DALYs were 810,000 and 20,578,000, respectively. The number of incident cases increased by 75% between 1990 and 2015, mostly because of changes in population age structures and population growth. Hepatitis B and hepatitis C viruses were responsible for 30% and 21% of liver cancer-related deaths, respectively. As prevention and treatment for risk factors evolve over time, the trend of liver cancer may shift, and we should adapt accordingly. Choi et al.⁶ reported that the crude numbers of DALY, mortality, and liver cancer increased during the study period, while the age standardization rates of DALY, mortality, and incidence decreased. The age-standardized rates of DALYs and mortality rates decreased from 258.4 to 151.1 and 8.9 to 5.9, respectively, owing to the aging population structure. This indicates that most liver cancers occur intensively in the oldest age groups. It is believed that it is time to call for a reasonable guideline for the surveillance and treatment strategy for liver cancer in the oldest, most
vulnerable population with risk factors. The burden of liver cancer varies worldwide. East Asia had the highest DALYs and death rates. Fortunately, both the DALYs and mortality rates in East Asia have decreased by approximately 60% over the past 20 years. In contrast, the age-standardized DALYs rates in North America and Australasia, regions with lower incidence and mortality, increased by 107% and 94.8%, respectively. In particular, in the high-income Asia-Pacific region, North America, and Australia, the age-standardized DALYs and mortality rates of liver cancer are increasing. Choi et al.\textsuperscript{6} meticulously assessed DALYs and mortality rates based on etiology, income level, sex, and age differences. Hepatitis B and C viruses remain the top causes of liver cancer DALYs and mortality, although the rates appear to be declining. This phenomenon might be due to the introduction of hepatitis B virus vaccines and antiviral therapies (nucleos(t)ides and direct-acting agents).\textsuperscript{8} Nonetheless, the burden of alcohol consumption and non-alcoholic fatty liver disease is increasing. This trend is evident in North America, Eastern Europe, and other high-income countries. Unlike viral hepatitis, the criteria for the high-risk group of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NASH)-related liver cancer have not yet been defined, and there is a lack of social consensus on how to screen, evaluate, and manage the so-called “high-risk group.”\textsuperscript{9,10} This indicates that some high-income countries require a national strategy or reasonable screening algorithm for non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NASH)-related liver cancer. Another interesting point is that alcohol-related liver cancer remains an important cause of liver cancer in almost all countries. The crude number of alcohol-related liver cancer cases increased, while the age-standardized incidence rates remained unchanged. Unlike other causes, it is an area of low social attention and pharmaceutical interest in the development of relevant medications and screening strategies for high-risk groups. This seems to be the greatest unmet social need for the future. These results may lead to the development of location-specific prevention and treatment strategies.

However, one important question was left unanswered in the current paper. Choi et al.\textsuperscript{6} performed a comparative analysis focusing on age-adjusted DALYs and mortality rates, but crude numbers were rarely presented. Therefore, it is still difficult to answer the question of how to distribute goods, and further economic evaluations are needed.

Over the next 20 years, the burden of primary liver cancer is expected to rise. Rumgay et al.\textsuperscript{11} extracted data from the GLOBOCAN 2020 web-based platform, presenting global cancer statistics. They predicted that the incidence of liver cancer would increase by 55.0% and the number of deaths would increase by 56.4% between 2020 and 2040. Therefore, primary liver cancer has always posed a significant threat to global health, both in the past and in the future.

The results of Choi et al.\textsuperscript{6} provide an unmet need for social consensus on the surveillance and management of liver cancer in the oldest population. In addition, the necessity of establishing a screening strategy for non-alcoholic fatty liver disease/NASH-related liver cancer, which is leading to an increasing liver cancer incidence in the future, was suggested. Finally, they also suggested a social agenda for the social burden of alcohol-related liver cancer, which has a relatively low social interest.

**Authors’ contributions**

JHO drafted the manuscript. DWJ reviewed and finalized the manuscript.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


**Abbreviations:**

DALY, disease-adjusted life year; NASH, non-alcoholic steatohepatitis


The current trends in the health burden of primary liver cancer across the globe

Peter Konyn1, Aijaz Ahmed2, and Donghee Kim2

1Department of Medicine, Stanford University School of Medicine, Stanford, CA; 2Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, CA, USA

Keywords: Hepatocellular carcinoma; Global burden of disease; Hepatitis

The face of primary liver cancer has evolved over the past century and is one of the most commonly diagnosed cancers in the world. Despite advancements in screening and treatment, primary liver cancer remains deadly, with global mortality rates closely matching incidence. According to data from Global Cancer Statistics 2020, liver cancer is the sixth most common type of cancer, yet it has the third highest mortality rate.1 There is a male predominance in the burden of primary liver cancer with a ratio of 2-3:1, such that primary liver cancer is the second leading cause of cancer-related mortality among men.1 Primary liver cancer may refer to any malignancies within the liver, including angiosarcoma or intrahepatic cholangiocarcinoma, though it usually refers to hepatocellular carcinoma (HCC), which makes up 75–85% of all cases.1 HCC typically develops in patients with liver cirrhosis, and the most important etiologies of underlying liver disease associated with the development of HCC are chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, alcohol-related liver disease (ALD), and non-alcoholic fatty liver disease (NAFLD).2 Incidence and mortality of primary liver cancer vary widely across the globe mainly due to variations in risk factor profiles and etiology from region to region (Fig. 1).1,2 Many nations have expanded programs for screening, treatment, and prevention of primary liver cancer and its predominant risk factors, which have already begun affecting trends in cancer incidence.3 For example, China reported a 65% reduction in HCC-related mortality between 1982 and 2009 after enacting agricultural policy reforms in the 1980s, which improved storage methods of rice and maize, subsequently reducing the burden of aflatoxin exposure in the country.4

In a recent issue of the Clinical and Molecular Hepatology, Choi and colleagues5 reported global trends in age-standardized rates of incidence, mortality, and disease-adjusted life years (DALYs) for primary liver cancer between 1990 and 2019 in order to better characterize the effect of targeted interventions at reducing the burden of primary liver cancer and its main risk factors. This study used data from the Global Burden of Disease (GBD) database from 1990 to 2019, which includes data from 204 countries and territories extracted from censuses, household surveys, civil registration and vital sta-
In this study, age-standardized rates of prevalence (10.2 [95% uncertainty interval, UI]: 9.2–11.3] to 9.1 [95% UI: 8.3–10.0]) and incidence (9 [95% UI: 8.1–10] to 6.5 [95% UI: 5.9–7.2]) showed a decline between 1990 and 2019. Similarly, global age-standardized DALYs and mortality rates decreased between 1990 and 2019 by 41.5% (95% UI: 31.5–49.8) and 33.4% (95% UI: 23.2–41.9), respectively. Most of this decline happened between 1996 and 2012, after which the age-standardized mortality rates remained stable without further decrease. Despite the decline in age-standardized data, crude numbers of DALYs and deaths from liver cancer increased over time. This effect is likely caused by a net improvement in disease outcomes alongside an increasing population of older adults. This study showed that HBV remains the leading cause of liver cancer mortality and incidence, followed by HCV, ALD, and NAFLD. In regions with a relatively high burden of HCC related to HBV, such as East Asia and Asia Pacific, age-standardized mortality and incidence rates were highest, though they experienced the most dramatic decrease over the study period. Conversely, age-standardized DALYs rates increased most dramatically in Cen-

Abbreviations:
HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; ALD, alcohol-related liver disease; NAFLD, nonalcoholic fatty liver disease; DALYs, disease-adjusted life years; GBD, Global Burden of Disease; UI, uncertainty interval; COVID-19, coronavirus disease 2019

tral Asia (150.2%), followed by North America (107%) and Australasia (94.8%), where rates of ALD and NAFLD are on the rise.

It is important to note that, though Western Sub-Saharan Africa and Southeast Asia had higher age-standardized DALYs rates in 2019 than East Asia (76.7 and 176.4 vs. 69.1, respectively), neither region experienced as drastic of a percent decrease between 1990 and 2019 as was seen in East Asia (-17.2% and -12.2% vs. -65.5%, respectively). This difference is likely related to variance in implementing national vaccination programs against HBV and accessing treatment with nucleos(t)ide analogue antivirals.3 After China adopted a universal immunization program against HBV in 1992, there was a decline in the seroprevalence of hepatitis B surface antigen in children under 5 years old, which fell to <1.0% by 2006.7 Taiwan, whose universal HBV vaccination program started 8 years earlier, experienced a similar effect.4 In contrast, only seven countries in Sub-Saharan Africa had formalized a plan to combat viral hepatitis by 2017, and only 10 of 47 countries in Africa included a birth dose in their routine immunization program.8

Additionally, the lack of nationalized HCC surveillance programs and lower access to healthcare resources result in higher rates of advanced-stage liver cancer or severe liver dysfunction at presentation in Sub-Saharan Africa.9 A multinational retrospective observational cohort study from 2016 showed that 72% of patients in Sub-Saharan Africa had Barcelona-Clinic Liver Cancer stage D HCC at the time of diagnosis, and only 3% would go on to receive any HCC-directed treatment.10 Even in high-income countries like the United States, racial and ethnic minorities experience disparities regarding HCC outcomes, as non-Hispanic Blacks are less likely to be diagnosed with early-stage disease.11 In addition, non-Hispanic Blacks experience 12% higher HCC-related mortality than non-Hispanic Whites, based on the data from the Surveillance, Epidemiology, and End Results program between 1995–2006.12

In this study, the regions with the highest age-standardized DALYs rate for HCV-associated liver cancer in 2019 were high-income Asia Pacific, North Africa, and Middle East.5 This reflects the HCV epidemics in Japan and Egypt that resulted from the use of shared needles as part of nationwide anti-schistosomal campaigns that started in the 1950s.4.13 In response to this epidemic, Egypt established a national program for screening and treating viral hepatitis and aimed to eliminate HCV by 2030.14 Other nations have made significant progress in controlling HCV as well. For example, age-standardized HCC-related mortality decreased in the United States by an annual rate of -3.5% (95% confidence interval, -5.9 to -1.1) between 2014 and 2018, reflecting the impact of direct-acting antiviral agents for the treatment of HCV.15

While national programs for screening, vaccination, and treatment of viral hepatitis played a prominent role in reducing age-standardized DALYs and mortality rates,16 the rates of ALD and NAFLD-related liver cancer are increasing at an alarming rate. Data from GBD do not even capture the anticipated rise in ALD and NAFLD due to increased alcohol consumption and rates of obesity observed during and after the coronavirus disease 2019 (COVID-19) pandemic.17 What is particularly alarming about this trend is how challenging it is to address these conditions compared to viral hepatitis. Unlike viral hepatitis, which can be cured or suppressed with oral antiviral agents, treatment of ALD and NAFLD requires multimodal and multidisciplinary approaches to treatment beyond medication.

This study skillfully utilized one of the only major multinational cancer databases to demonstrate trends in primary liver cancer across the globe, including the patient-centered composite metric of DALYs. Most limitations of the study pertain directly to the source data. While both GBD and Globocan are established and well-designed, they take different approaches to data collection. Globocan prioritizes data from national registries. This strategy results in high-quality inputs when data is available, but many low-income countries do not have national cancer registries.18 Globocan works around this by estimating a country’s cancer burden by extrapolating data from neighboring countries, which may have drastically different geopolitical influences affecting the epidemiology of certain liver cancer risk factors. When national registries are not available for a population, GBD uses alternative sources of information, such as surveys or verbal autopsies. Because of these heterogeneous approaches, noticeable discrepancies have been observed between the two databases. For example, age-standardized incidence rates of lip and oral cancers dramatically differed between Globocan 2020 and GBD 2019 for Papua New Guinea, Vietnam, China, Pakistan, and Indonesia, likely because these countries did not have quality data regarding lip and oral cancers in their national registries.19 Additionally, the data in question is now more than 3 years old. Though the most recent iteration of Globo-
can includes data from 2020, both major data sets have yet
to capture any effects of the recent COVID-19 global pan-
demic. Lastly, these databases describe cancer in terms of
site of occurrence. Since the different types of primary liver
cancer have different risk factors, it would be helpful to be
able to examine trends specifically for HCC, separate from
other primary liver cancers.

In summary, Choi and colleagues\textsuperscript{5} utilize cancer epidemi-
ological data from the large, multinational GBD 2019 database
to demonstrate that age-standardized rates of DALYs, mortal-
ity, incidence, and prevalence declined from 1990 to 2019.
While HBV remains the world’s predominant risk factor for
liver cancer, the global burden of viral hepatitis is decreasing,
while ALD- and NAFLD-related liver cancer is on the rise. The
necessary next steps should be identifying how the COVID-19
pandemic and the continued advancement of treatment
strategies for chronic liver disease have altered these trends.

Author’s contribution

Dr. Peter Konyn was involved in the study concept and de-
sign, acquisition of data, interpretation of data, and drafting
of the manuscript. Dr. Aijaz Ahmed and Dr. Donghee Kim
were involved in the study concept and design, interpreta-
tion of data, drafting of the manuscript, critical revision of the
manuscript, and study supervision.

Acknowledgements

The work had no funding sources, which could have influ-
enced the study design and conduct, analysis, interpretation
of the data, review, and approval of the manuscript.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jem-
of incidence and mortality worldwide for 36 cancers in 185
2. Konyn P, Ahmed A, Kim D. Current epidemiology in hepatocel-
lar carcinoma. Expert Rev Gastroenterol Hepatol 2021;15:1295-
1307.
3. Korean Liver Cancer Association (KLCA) and National Cancer
Center (NCC) Korea. 2022 KLCA-NCC Korea practice guidelines
for the management of hepatocellular carcinoma. Clin Mol
Hepatol 2022;28:583-705.
4. Goh GB, Chang PE, Tan CK. Changing epidemiology of hepa-
tocellular carcinoma in Asia. Best Pract Res Clin Gastroenterol
2015;29:919-928.
burden of primary liver cancer and its association with underly-
ing aetiologies, sociodemographic status, and sex differences,
1990-2019: a DALYs-based analysis of the Global Burden of Dis-
6. GBD 2019 Diseases and Injuries Collaborators. Global burden of
369 diseases and injuries in 204 countries and territories, 1990-
2019: a systematic analysis for the Global Burden of Disease
2020;396:1562.
serosurvey of hepatitis B in China--declining HBV prevalence
8. Okeke E, Davwar PM, Roberts L, Sartorious K, Spearman W, Malu
A, et al. Epidemiology of liver cancer in Africa: Current and fu-
9. Kedar Mukthinuthalapati VVP, Sewram V, Ndlovu N, Kimani S,
Abdelaziz AO, Chiao EY, et al. Hepatocellular carcinoma in Sub-
10. Yang JD, Mohamed EA, Aziz AO, Shousha Hl, Hashem MB, Na-
beel MM, et al.; Africa Network for Gastrointestinal and Liver
Diseases. Characteristics, management, and outcomes of pa-
tients with hepatocellular carcinoma in Africa: a multicountry
observational study from the Africa Liver Cancer Consortium.
Gastroenterol Hepatol 2022;7:704.
11. Rich NE, Hester C, Odewole M, Murphy CC, Parikh ND, Marrero
JA, et al. Racial and ethnic differences in presentation and out-
comes of hepatocellular carcinoma. Clin Gastroenterol Hepatol
2019;17:551-559.e1.
12. Mathur AK, Osborne NH, Lynch RJ, Ghaferi AA, Dimick JB,
Sonnenday CJ. Racial/ethnic disparities in access to care and
survival for patients with early-stage hepatocellular carcinoma.
tis C infection in Egypt: prevalence, impact and management
2017;9:35.
14. Hassanin A, Kamel S, Waked I, Fort M. Egypt’s ambitious strat-


The imitator of immune-tolerant chronic hepatitis B: A killer in disguise

Moon Haeng Hur and Jeong-Hoon Lee
Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

Keywords: Hepatitis B virus; Indolent immune clearance phase; Surveillance; Antiviral treatment; Liver biopsy

Chronic hepatitis B (CHB) is the leading cause of hepatocellular carcinoma (HCC), especially in the Asia-Pacific region. For CHB patients in the clinical immune-tolerant (IT) phase or HBeAg-positive chronic hepatitis B virus (HBV) infection phase, the Asian Pacific Association for the Study of the Liver and the Korean Association for the Study of the Liver guidelines recommend considering liver biopsies to determine antiviral treatment if there are risk factors. The guidelines from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) suggest that antiviral treatment may be initiated in selected patients based on their age and/or the histological degree of hepatic fibrosis and inflammation. Despite a number of debates in terms of the prevention for liver-related events, there may be general agreement that antiviral therapy is unlikely to be beneficial for patients in the “true” IT phase of CHB, which can be identified by the absence of both active hepatic inflammation (which reflects current hepatic injury) and significant hepatic fibrosis (which reflects previous liver injury). Therefore, it is necessary to define the true IT phase succinctly in everyday practice to optimize the management of CHB patients.

In clinical practice, however, it may be challenging to clearly distinguish between the “true” IT phase and the “fake” IT phase (or indolent immune-active phase) because blood biochemical assays and imaging examinations are not sensitive enough to capture active inflammation and significant hepatic fibrosis, respectively, without histological evaluation. Several lines of evidence suggest that the clinical IT phase (HBeAg-positive, high HBV DNA level, and normal ALT) may not necessarily represent the true IT phase. In a study involving Asian-American CHB patients in the clinical IT phase, approximately one-fourth of the patients exhibited histologically significant fibrosis (F2 or F3). A study conducted in Hong Kong showed that 10% of CHB patients with normal ALT had advanced fibrosis on transient elastography. Moreover, surprisingly, in the article that accompanies this editorial, Yoo et al. reported that 177 out of 259 (68.3%) patients who were in the clinical IT phase (defined by HBeAg-positive, HBV DNA level of ≥6 log10 IU/mL, and ALT level of ≤60 U/L) were not in the true histological IT phase (defined by both hepatic fibrosis and inflammation grades of 0 or 1): 174 pa-
tients had significant fibrosis ranging from F2 to F4, and 94 patients had moderate or severe inflammation. However, an unexpectedly higher proportion of the fake IT phase in the current study, compared to prior studies, needs to be interpreted with caution. Instead of performing liver biopsy on all consecutive patients in the clinical IT phase, it was left to the discretion of attending physicians, which might be influenced by several hepatic fibrosis-related indicators (e.g., platelet counts and serum albumin level). Under this condition, the proportion of patients belonging to the fake IT phase may be overestimated. In fact, patients in the fake IT phase had lower platelet counts and serum albumin levels than those in the true IT phase. Based on the clinical outcomes of patients in the true vs. fake IT phase, the authors suggested that age over 35, high aspartate aminotransferase (AST), and low albumin levels could be useful in ruling out the histological IT phase.

Specifically, age was identified as a risk factor for liver-related events independent of histological fibrosis grade, supporting the recommendations of AASLD/EASL guidelines, which suggest the initiation of antiviral treatment among CHB patients in the clinical IT phase if the age exceeds 30–40 years. Even though the majority of the Korean CHB patients are infected with genotype C HBV and have a longer IT phase than patients from other regions, the results of this study suggest that the AASLD/EASL recommendation can be applied to Korean CHB patients as well.

In a Korean study, patients with IT phase CHB were reported to have a higher risk of HCC and death compared to those with immune-active phase CHB who were treated with antiviral agents. The IT group had 2.54- and 3.38-fold higher risks of HCC and death or transplantation, respectively, than treated immune-active phase patients. On the other hand, a recent meta-analysis showed comparable risks of HCC and mortality between the untreated IT and the treated immune-active phases among HBeAg-positive CHB patients without cirrhosis. Another retrospective study reported a negligible incidence of HCC among CHB patients in the IT phase if their fibrosis-4 index is low. This discrepancy may result from the inadequacy of current clinical criteria for the IT phase.

Another Korean study, including CHB patients with ALT below twice the upper limit of normal, demonstrated that the

**Figure 1.** Clinical significance of fake IT phase. Patients in the fake IT phase had a higher risk of liver-related events (liver cirrhosis and HCC) than those in the true IT phase. Age, HBV DNA load, platelet count, and serum levels of albumin or AST can be used to suspect the presence of the fake IT phase. AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; AST, aspartate aminotransferase; EASL, European Association for the Study of the Liver; HCC, hepatocellular carcinoma; IT, immune-tolerant; KASL, Korean Association for the Study of the Liver.

**Abbreviations:**
AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; EASL, European Association for the Study of the Liver; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IT, immune-tolerant; TDF, tenofovir disoproxil fumarate
risk of HCC was highest with HBV DNA levels of 6–7 log$_{10}$ IU/mL and lowest with >8 log$_{10}$ IU/mL, in contrast to the findings of the REVEAL cohort, which showed that HCC risk increased proportionally with HBV DNA level. This difference may stem from the fact that the REVEAL cohort included more HBeAg-negative patients and HBV DNA level >6 log$_{10}$ IU/mL was not further stratified. Theoretically, prolonged HBV infection may lead to the expansion of HBV-resistant hepatocyte clones, which can reduce HBV replication but promote HCC development. Consequently, HBeAg-positive patients with relatively low levels of HBV DNA (6–7 log$_{10}$ IU/mL) may experience poor clinical outcomes, and the presence of the fake IT phase should be suspected in these patients. In the accompanying study by Yoo et al., HBV DNA level was not identified as a factor to rule out histological IT phase. This may be due to the relatively low proportion of patients (12.7%) with HBV DNA levels of 6–7 log$_{10}$ IU/mL. Nonetheless, it is noteworthy that HBV DNA levels of 6–7 log$_{10}$ IU/mL increased the odds of both being in the fake IT phase (odds ratio=1.96, $P=0.3$) and having advanced fibrosis (odds ratio=1.93, $P=0.068$), although the results failed to reach statistical significance.

The results of current and previous studies collectively indicate that at least 10–30% of patients classified as the clinical IT phase may not be in the true IT phase. Clinical diagnosis of the IT phase may be inaccurate, and patients in the fake IT phase can be potential candidates for antiviral treatment. However, several factors should be considered before the initiation of antiviral therapy in CHB patients with high HBV DNA and normal ALT levels. First, under suboptimal activation of the host immune system, antiviral therapy can lead to low viral responses. In a prospective study involving HBeAg-positive patients with normal levels of ALT and high levels of HBV DNA, tenofovir disoproxil fumarate (TDF) with and without emtricitabine showed 76% and 55% of complete virologic response rates, respectively, at week 192. Fortunately, however, a Korean multicenter retrospective study revealed that the majority of patients achieved complete virologic response after 7–8 years of antiviral treatment. Although several patients infected by TDF-resistant HBV have been confirmed in Korea, all patients had a preexisting multidrug-resistance mutation, and none were initially treated with antivirals with a high genetic barrier. Second, long-term side effects of antivirals, such as distinct renal and bone toxicity during TDF treatment, should be considered. Although tenofovir alafenamide has been introduced and shown to be safe for the kidney and bone, it is suspected to be related to weight gain and dyslipidemia. Third, a well-designed prospective study should confirm whether antiviral treatment can reduce the risk of liver-related outcomes among CHB patients in the fake IT phase. Currently, a multinational randomized-controlled trial (ClinicalTrials.gov identifier: NCT03753074), which investigates the effect of tenofovir alafenamide treatment on the incidence of liver-related events, including HCC, in CHB patients with intermediate-to-high levels of HBV DNA (4–8 log$_{10}$ IU/mL), normal ALT, and an age of ≥40 years, is ongoing, and the results are awaited.

A recent Korean nationwide cohort study demonstrated that untreated CHB patients had a higher risk of extrahepatic malignancies, which was normalized with antiviral treatment. Future antiviral therapy should be determined based on the expected results of both liver-related (liver cirrhosis, HCC, liver-related death, and liver transplantation) and non-liver-related outcomes (side effects and extrahepatic malignancy risk). Antiviral treatment indication is expected to be expanded, but it is necessary to begin with patients who are obviously at high risk. In this sense, patients in the fake IT phase may be suitable next targets. As reported in the accompanying article (Fig. 1), when a patient has any risk factors (e.g., age of >35 years, intermediate HBV DNA level [6–7 log$_{10}$ IU/mL, and low platelet count), clinicians should heighten their suspicion if he or she is in the fake IT phase, a silent killer in disguise.

**Authors’ contribution**
Conceptualization, J-H Lee; Original draft, MH Hur and J-H Lee; Review and editing, MH Hur and J-H Lee.

**Conflicts of Interest**
MH Hur has no conflict of interest to disclose. J-H Lee receives research grants from Yuhan Pharmaceuticals and GreenCross Cell, and lecture fees from GreenCross Cell, Dae-woong Pharmaceuticals, and Gilead Korea.

**REFERENCES**


Is liver biopsy essential to identifying the immune tolerant phase of chronic hepatitis B?

Joo Hyun Oh¹ and Dong Hyun Sinn²

¹Department of Medicine, Nowon Eulji Medical Center, Eulji University School of Medicine; ²Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Keywords: Hepatitis B, chronic; Biopsy; Fibrosis

The natural history of chronic hepatitis B virus (HBV) infection is complex; it is characterized by different immune phases that may overlap.¹ The first phase is an immune-tolerant phase that is considered to be essentially benign in nature.² This phase is characterized by hepatitis B e antigen (HBeAg) positivity, very high levels of HBV DNA, and persistent normal alanine aminotransferase (ALT) levels.³ However, the notion that the immune-tolerant phase is truly benign in nature has been challenged.⁴ In addition, it is often difficult to accurately diagnose the true immune-tolerant phase.⁵ The criteria and terminology used to define this phase also vary across international guidelines. The American Association for the Study of Liver Disease (AASLD) guidelines define immune-tolerant phase by HBeAg positivity, high serum HBV DNA levels (>10⁶ IU/mL), and normal serum ALT level (<33 U/L for males and <25 U/L for females).⁵ The European Association for the Study of the Liver (EASL) guidelines define immune-tolerant phase by HBeAg positivity, high serum HBV DNA level (>10⁷ IU/mL), and normal serum ALT level (<34 U/L for males, <30 U/L for females) (Table 1).⁶ The Asia Pacific Association for the Study of the Liver (APASL) guidelines provide similar criteria without specific HBV DNA or ALT cut-off values.⁷ The Korean Association for the Study of the Liver (KASL) guidelines define the immune-tolerant phase by HBeAg positivity, high serum HBV DNA level (>10⁷ IU/mL), and normal serum ALT level (<34 U/L for males, <30 U/L for females) (Table 1).⁸

HBV is a non-cytopathic virus that does not directly cause hepatocyte death.⁴ Liver injury is considered immune mediated.⁴ The hallmark of the immune-tolerant phase is minimal or absent liver injury.⁷ Hence, there should be no necroinflammation or liver fibrosis on histologic exam in an immune-tolerant phase.⁹ As a highly sensitive marker of liver injury, serum ALT level is often elevated when liver injury is present.¹⁰ In the immune-tolerant phase, ALT level should not be elevated. However, the cutoff used to define ‘normal’ serum ALT levels differs among international guidelines (Table 1). ALT is also an imperfect marker of liver injury. Studies have shown that ALT is an inadequate marker to correctly identify the immune-tolerant phase.¹¹ Thus, whether liver biopsy is necessary to correctly identify the immune-tolerant phase
In this issue of the Clinical and Molecular Hepatology, Yoo et al. studied 259 CHB patients who underwent liver biopsy with an immune-tolerant phase that was serologically identified. Surprisingly, they found that only 82 (31.7%) of these patients were in an immune-tolerant phase based on histologic findings. The authors used a serum HBV DNA level cutoff of >10^6 IU/mL and serum ALT level cutoff of <60 IU/L for both sexes to identify an immune-tolerant phase. Such criteria were less stringent than the AASLD or the EASL criteria. This might have contributed to the high proportion of patients in the “grey zone”, a territory that does not fall into typical CHB clinical phases. For patients who fall into this zone, the extent of liver damage and prognosis are uncertain. It is important to note that the ability to differentiate between immune-tolerant phase and other phases may be impacted by the inclusion of “grey zone” patients in the analysis. However, even when the more stringent AASLD or EASL criteria were used to analyze the same patients, only 31.7% or 34.0% of serologically immune-tolerant patients were in the same phase based on histological findings. This suggests that relying solely on the clinical definition may not accurately predict the severity of liver damage in CHB patients. Therefore, it is necessary to perform liver biopsy to accurately identify patients who require close monitoring or antiviral therapy.

In this cohort, fibrosis-4 index (FIB-4) was not as good indicator, as previously reported. In patients with a low FIB-4 index (<1.45), 18% had advanced fibrosis (≥F3). The risk of liver-related events such as hepatocellular carcinoma, liver cirrhosis, liver transplantation, and death was significantly lower for patients in a histologically immune-tolerant phase. These findings suggest that liver biopsy might be necessary to identify those who are in a true immune-tolerant phase and show a benign clinical course. Nevertheless, liver biopsy is an invasive procedure with potential complications. Performing liver biopsy for all patients who are presumed to be in an immune-tolerant phase is unrealistic. The authors thus suggested an age cut-off of 35 years or older as a criterion when considering liver biopsy. However, questions remain due to the invasiveness of liver biopsy. What if liver biopsy reveals a histologically immune-tolerant phase for those who are aged 35? Do they need repeat liver biopsy as they age to determine whether they are still in the histologically immune-tolerant phase? If repeat biopsy is necessary, how often should it be performed? From this perspective, there should be other criteria, beyond age, to define a true immune-tolerant phase, which should be essentially benign in nature. Novel biomarkers, which can now be measured using blood, might play a role in defining disease phases. Some potential biomarkers that have been investigated include serum cytokines, specific T-cell subsets, microRNA, and hepatitis B core-related antigen (HBCrAg). For instance, patients in an immune-tolerant phase have significantly higher levels of HBCrAg than those who are in an immune clearance phase or an inactive carrier phase. Although these biomarkers alone cannot definitively identify the immune-tolerant phase in CHB patients, they might be a useful tool for predicting the immune-tolerant phase when they are used in combination with other clinical and laboratory parameters. As performing repeat liver biopsy to verify an immune-tolerant phase is unrealistic in clinical practice, studies using new novel non-invasive biomarkers are eagerly awaited.

Yoo et al. performed a retrospective study at academic institutions, and thus selection and/or indication bias might help explain the high incidence of necroinflammation or fibrosis in the immune-tolerant phase. Nevertheless, this study provides a strong and clear message. Patient should not be considered to be in a ‘genuine’ immune-tolerant phase without liver biopsy. Due to several limitations, liver biopsy cannot be recommended for all patients who are presumed to be in an immune-tolerant phase in clinical practice. However, this study revealed that, without such histologic information, patients presumed to be in an immune-tolerant phase must be carefully evaluated using a variety of factors.

Author’s contribution
JHO drafted the manuscript. DHS reviewed and finalized the manuscript.

Abbreviations:
HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; AASLD, American Association for the Study of Liver Disease; EASL, European Association for the Study of the Liver; APASL, Asia Pacific Association for the Study of the Liver; KASL, Korean Association for the Study of the Liver; FIB-4, fibrosis-4, HBCrAg, hepatitis B core-related antigen

https://doi.org/10.3350/cmh.2023.0054
Table 1. Criteria for an immune-tolerant phase of chronic hepatitis B according to different guidelines

<table>
<thead>
<tr>
<th>Features</th>
<th>AASLD⁵</th>
<th>EASL⁶</th>
<th>APASL⁷</th>
<th>KASL⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminology</td>
<td>IT phase</td>
<td>HBeAg positive chronic infection</td>
<td>IT phase</td>
<td>IT phase</td>
</tr>
<tr>
<td>HBeAg</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>&gt;10⁶ IU/mL</td>
<td>&gt;10⁶ IU/mL</td>
<td>Active HBV replication</td>
<td>&gt;10⁶ IU/mL</td>
</tr>
<tr>
<td>ALT</td>
<td>Normal ALT (&lt;33 U/L for males, &lt;25 U/L for females)</td>
<td>Persistently normal ALT (&lt;40 U/L)</td>
<td>Normal ALT</td>
<td>Persistently normal ALT (&lt;34 U/L for males, &lt;30 U/L for females)</td>
</tr>
<tr>
<td>Fibrosis/inflammation</td>
<td>None/minimal</td>
<td>None/minimal</td>
<td>None</td>
<td>None/minimal</td>
</tr>
<tr>
<td>Fibrosis assessment</td>
<td>Consider noninvasive methods (liver stiffness measurement, APRI, or FIB-4) or liver biopsy if persistent borderline normal or slightly elevated ALT, Age &gt;40 years of age</td>
<td>A liver biopsy or a non-invasive test if elevated ALT</td>
<td>Consider biopsy if noninvasive tests suggest evidence of significant fibrosis, ALT persistently elevated, Age &gt;35 years, or family history of HCC/cirrhosis</td>
<td>Consider biopsy if persistently elevated ALT, Age &gt;35–40 years</td>
</tr>
</tbody>
</table>

AASLD, American Association for the Study of Liver Diseases; EASL, European Association for the Study of the Liver; APASL, The Asian Pacific Association for the Study of the Liver; KASL, The Korean Association for the Study of the Liver; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; IT, immune-tolerant.
Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

Changing the nomenclature from nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease is more than a change in terminology

Eileen Laurel Yoon\textsuperscript{1,2} and Dae Won Jun\textsuperscript{1,2}

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

\textbf{Keywords:} Non-alcoholic fatty liver disease; Metabolic syndrome; Terminology; Clinical trial

In this issue of \textit{Clinical and Molecular Hepatology}, Gofton et al.\textsuperscript{1} reviewed the difference between metabolic dysfunction-associated fatty liver disease (MAFLD) and non-alcoholic fatty liver disease (NAFLD). Since 2020, MAFLD has been proposed as a term referring to fatty liver diseases associated with metabolic dysfunction, as a replacement for the term NAFLD, which is based on negative diagnostic criteria.\textsuperscript{2} MAFLD has subsequently been endorsed by several societies specializing in the study of liver diseases.\textsuperscript{3,4} However, a consensus has not yet been reached across a significant number of key national and pan-national societies, and a consensus with broader global multi-stakeholders is required.

The change of nomenclature from NAFLD to MAFLD has several advantages; it raises awareness of the disease in patients and primary care physicians, clarifies treatment strategies, and enables a holistic approach to treating patients with liver disease.\textsuperscript{5} First, MAFLD allows better recognition of patients with a more advanced stage of hepatic fibrosis and greater risk of overall mortality.\textsuperscript{6-8} Second, MAFLD enables improved management of patients with comorbid liver diseases other than NAFLD. In the era of NAFLD, patients with chronic hepatitis B were classified as such regardless of presence of hepatic steatosis. Thus, the importance of lifestyle modifications in these patients has been underestimated. However, there is growing evidence that comorbid hepatic steatosis worsens the prognosis in patients with chronic viral hepatitis.\textsuperscript{9-11} In this regard, MAFLD enables multidisciplinary treatment for such patients. Non-alcoholic-, alcohol-associated-, and viral hepatitis-steatotic liver disease will be discussed in the planned consensus meeting. These novel terms not only acknowledge the dual etiology of fatty liver disease, but also increase awareness of the disease.\textsuperscript{12} Third, MAFLD emphasizes metabolic dysfunction as the basic mechanism of fatty liver disease, both through its name and the inclusive diagnostic criteria.\textsuperscript{7} This change in name also would allow in-
tuitive explanation of causes and treatment approaches to patients. Additionally, it could reduce the time from diagnosis to treatment by omitting the need to exclude other liver diseases during diagnosis.

The change of nomenclature from NAFLD to MAFLD is more than a simple change in terminology and will have an extensive impact on research, the pharmaceutical industry, insurance companies, and government policies. The change in nomenclature to “MAFLD” requires significant changes in ongoing NAFLD clinical trial designs, primary endpoints, clinical outcomes of final approval, and therapeutic targets of treatment due to the new inclusion criteria.

There are several reasons to wait for a robust consensus on the nomenclature change among the broader body of stakeholders, including pharmaceutical companies, authorities, and various patient alliances. First, the heterogeneous aspect of NAFLD is overlooked in MAFLD. In early clinical trials, researchers focused on controlling insulin resistance or metabolic risk factors, as NAFLD was deemed a manifestation of metabolic syndrome in the liver. However, most clinical trials with insulin sensitizers, lipid-lowering agents, and anti-obesity treatments have not been successful in NAFLD treatment. The development of fatty liver disease is based on heterogeneous mechanisms and is more complex than originally believed. Thus, an excessive focus on metabolic dysfunction could veil novel therapeutic targets and delay drug development. Genetic factors, intestinal dysbiosis, and sarcopenia, which are not closely related to metabolic dysfunction as to NAFLD, are underestimated pathophysiological mechanisms in MAFLD. Nonetheless, these factors contribute to the development of NAFLD and are possible starting points for drug development. Second, the new definition of MAFLD may increase the heterogeneity of the target population during phase III clinical trials, as it also includes individuals with viral hepatitis or alcoholic liver disease. Controlling the effects of viral hepatitis and alcohol consumption is a complex problem. Third, the use of MAFLD resolution as a primary endpoint in clinical trials may lead to ambiguity. Currently, nonalcoholic steatohepatitis resolution without exacerbation of liver fibrosis is used as an endpoint in clinical trials for NAFLD. However, the endpoint in MAFLD would be different from the endpoint currently used in NAFLD. Therefore, long-term data are needed to determine whether improvement in metabolic dysfunction or normalization of bodyweight could be viewed as MAFLD resolution when it is achieved without histological improvement. Fourth, it may be difficult to evaluate the efficacy of candidate drugs in clinical trials when these drugs target inflammation or fibrosis without ameliorating metabolic abnormalities. A considerable number of candidate drugs under development is unrelated to metabolic improvement or weight loss.

In conclusion, we propose a cautious and in-depth discussion to reach a consensus among all stakeholders before the terminology is changed from NAFLD to MAFLD.

Authors’ contribution
YEL, first drafting and revision of the manuscript; JDW, organized and supervised the manuscript. All the authors approved the final manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
5. Kawaguchi T, Tsutsumi T, Nakano D, Eslam M, George J, Torimu...
The growing burden of non-alcoholic fatty liver disease on mortality

Ju-Yeon Cho¹ and Won Sohn²

¹Department of Internal Medicine, College of Medicine, Chosun University, Gwangju; ²Division of Gastroenterology, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea

Keywords: Nonalcoholic fatty liver disease; Global burden of disease; Mortality

Non-alcoholic fatty liver disease (NAFLD) is characterized by fat infiltration of the liver in the absence of significant alcohol intake, viral hepatitis, medications that may cause fatty liver, or other obvious causes. According to a meta-analysis, the annual incidence rate of NAFLD in Asia was 50.9 cases per 1,000 person-years, while its prevalence in Asia was 29.6%. The reported global prevalence of NAFLD is 30%, and it is rapidly increasing with a predicted rate of 50% of the population by 2040. The overbearing burden of the disease requires an increased understanding of the disease.

In this issue of Clinical and Molecular Hepatology, Konyn et al. have presented the results of an extensive review of the current literature, focusing on the causes and risk profiles of mortality in patients with NAFLD. Based on their findings, the authors have concluded that 1) NAFLD per se may not independently increase the risk of all-cause mortality; 2) the most common causes of death in patients with NAFLD include cardiovascular disease, extra-hepatic cancer, liver disease, and diabetes; 3) risk factors for increased mortality in NAFLD are mutation in the patatin like phospholipase domain containing 3 (PNPLA3) gene, low thyroid function, and sarcopenia; and 4) dietary modification and physical activity leading to weight loss have a significant effect on decreasing the mortality.

In the aforementioned review, the notion that the risk of NAFLD independently increases all-cause mortality was negated by a study by Kim et al., wherein metabolic-associated fatty liver disease (MAFLD), but not NAFLD, was associated with increased all-cause mortality after adjusting for metabolic risk factors. Another review by Ng et al. stated that all-cause mortality and cardiovascular mortality may be higher in MAFLD when compared with NAFLD. However, a recent study by Younossi et al. reported that all-cause mortality and cause-specific mortality in patients with NAFLD and MAFLD were not significantly different. de Avila et al. stated that NAFLD was independently associated with increased all-cause mortality. Another recent meta-analysis by Fu et al. reported that NAFLD was asso-
ciliated with an increased risk of all-cause mortality but not with liver-related mortality. The authors attributed this finding to a low rate of fibrosis in the community. These differences in the results may be due to the broad range of accepted definitions of NAFLD, since the individual studies used ultrasonography, transient elastography, or fatty liver index for diagnosis. Further studies are required to determine whether NAFLD is an independent risk factor for increased all-cause mortality.

Generally, cause-specific mortality associated with NAFLD is attributed to cardiovascular disease, extrahepatic cancer, and liver disease. In the review by Konyn et al., an emphasis on the impact of diabetes on NAFLD is noteworthy, since mortality among patients with diabetes and chronic liver disease is increasing, while age-standardized mortality due to diabetes is declining. According to the authors’ recommendations, clinicians should be cognizant of the bidirectional impact of NAFLD and diabetes.

Konyn et al. stated that the risk factors for increased mortality in patients with NAFLD include mutation in the PNPLA3 gene, low thyroid function, and sarcopenia. Another recent review regarding risk factors for the development and progression of NAFLD included not only mutations in the PNPLA3 gene and sarcopenia but also obesity, diet, type 2 diabetes mellitus, obstructive sleep apnea, and gut microbiome. The proposed factors contributing to the progression of the disease warrant further studies discussing the impact of mortality in patients with NAFLD.

The degree of fibrosis is a well-established risk factor predicting the mortality of NAFLD patients. Recent advances in stratification of fibrosis incorporating artificial intelligence, machine learning, and deep learning to the gold diagnostic standard of liver biopsy is reported to have increased the prediction of mortality. The diagnostic power of noninvasive tests including blood test panels (fibrosis-4 [FIB-4] index or NAFLD fibrosis score), transient elastography, and magnetic resonance elastography (MRE) have been studied and compared to liver biopsy. The use of more than 2 noninvasive tests (MEFIB [MRE plus FIB-4] or FAST [FibroScan-AST] for detecting significant fibrosis) to increase the yield of the diagnostic power for fibrosis have recently been reported. Further studies in predicting the mortality of NAFLD incorporating newly developed methods in diagnosing and stratifying fibrosis is warranted.

Lifestyle modifications, including physical activity and dietary modifications, are the cornerstones of therapy in patients with NAFLD to achieve a weight loss of ≥5% of total body weight for improvement in NAFLD, ≥7% for resolution of non-alcoholic steatohepatitis, and ≥10% for regression/stability of fibrosis. Diets including a high volume of whole and unprocessed foods, fiber, and unsaturated fats are recommended, with restrained consumption of red and processed meats, refined carbohydrates, and saturated fat.

The disease burden of NAFLD is growing in all-cause mortality and liver-related mortality. The importance of risk factors on mortality in NAFLD cannot be stressed enough. With the recent advances in genetics and diagnostic tools, the better prediction on the prognosis of NAFLD can be possible. Additionally, we may expect the reduction of mortality in NAFLD if new therapeutics is established beyond life style modification and currently available drugs in the near future.

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

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
2. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in...


Lean vs. obese phenotypes of nonalcoholic fatty liver disease: similar or different?

Ho Soo Chun\textsuperscript{1,2} and Minjong Lee\textsuperscript{1,2}

\textsuperscript{1}Department of Internal Medicine, Ewha Womans University College of Medicine; \textsuperscript{2}Department of Internal Medicine, Ewha Womans University Medical Center, Seoul, Korea

Keywords: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Obesity; Metabolic dysfunction; Mortality

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide, and its incidence and related complications are expected to rise with the global increase in metabolic disorders.\textsuperscript{1} Traditionally, NAFLD has been closely associated with obesity and metabolic dysfunction, including insulin resistance, abnormal lipid profiles, and fatty acid cytotoxicity.\textsuperscript{2} Despite the close association between NAFLD and obesity, NAFLD is increasingly being identified in non-obese populations.\textsuperscript{3} A recent systematic review and meta-analysis reported that around 40% of the global NAFLD population was classified as non-obese (body mass index [BMI] <30 kg/m\textsuperscript{2} in non-Asians; 25 kg/m\textsuperscript{2} in Asians) and almost a fifth as lean (BMI <25 kg/m\textsuperscript{2} in non-Asians; <23 kg/m\textsuperscript{2} in Asians).\textsuperscript{3} Clinical outcomes, such as the severity of metabolic dysfunction or the incidence of severe liver disease or mortality, of non-obese or lean individuals with NAFLD compared with obese individuals with NAFLD remain unclear due to variation across recent studies.

A recent meta-analysis of NAFLD reported that the degree of metabolic dysfunction was weight-dependent, with significantly less metabolic dysfunction in lean subjects compared with their overweight counterparts.\textsuperscript{4} Another systematic review showed that the incidence (per 1,000 person-years) of all-cause (12.1 vs. 7.5), liver-related (4.1 vs. 2.4), and cardiovascular mortality (4.0 vs. 2.4) was relatively higher, and that of new-onset cardiovascular disease (CVD) was lower (18.7 vs. 33.3) in non-obese or lean patients with NAFLD than in obese patients with NAFLD.\textsuperscript{5} In contrast, a recent longitudinal cohort study of 646 patients with biopsy-proven NAFLD reported that compared to non-lean patients with NAFLD (n=523, 81%), lean patients with NAFLD (n=123, 19%) with a low severity of liver fibrosis had no increased risk for overall mortality, but did face increased risk of developing severe liver disease (hazard ratio=2.69) during a mean follow-up of 19.9 years.\textsuperscript{5}

In the current issue of Clinical and Molecular Hepatology, Chan made some points about the possible reasons for these disparities.\textsuperscript{6} As mentioned in the review,\textsuperscript{6} studies may be confounded by selection bias. Lean patients with NAFLD ob-
served in secondary or tertiary clinical settings and diagnosed via liver biopsy may have more severe liver disease than expected. In addition, Chan noted the lack of consideration for important confounding factors, such as changes in alcohol consumption and body weight over time. Although accurate assessment of alcohol consumption is a prerequisite to accurate diagnosis and treatment of patients with NAFLD, unreported alcohol consumption can play a role for NAFLD progression and can vary during follow-up. Indeed, in a recent prospective observational study of 114 patients with NAFLD, repeated moderate to excessive alcohol consumption was detected in 28.6% of patients with presumed NAFLD who were at risk of alcohol-related liver damage. Interestingly, patients with repeated moderate or excessive alcohol consumption had a significantly lower BMI and fewer metabolic comorbidities. This suggests the importance of accurate assessment of alcohol intake when evaluating the prognosis of non-obese or lean patients with NAFLD.

Changes in body weight during follow-up could affect sarcopenia along with aging in patients with NAFLD. A recent large cohort study of 52,815 adult participants reported that 5-year changes in appendicular skeletal muscle mass were significantly higher in participants with NAFLD than in those without NAFLD (-281.3 g vs. -225.2 g). Furthermore, muscle loss was much faster in participants with NAFLD and significant liver fibrosis than in those without. Therefore, it may be the influence of sarcopenia that non-obese or lean patients with NAFLD have a poor prognosis, although they have fewer metabolic dysfunctions. As such, an appropriate assessment of sarcopenia is important in clinical studies. In addition, the etiology of NAFLD in lean individuals may be based on central obesity and visceral fat. Accordingly, the body weight-based BMI-driven approach for the classification of NAFLD may need to be reappraised.

In recent years, a number of genomic studies showed that genetic polymorphisms in the several genes, such as patatin-like phospholipase domain-containing-3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2), can be a major genetic determinant of NAFLD and its severity. A recent population-based study of 904 Asian subjects diagnosed with hepatic steatosis with proton-magnetic resonance spectroscopy reported that lean individuals were more likely to have a PNPLA3 gene polymorphism than overweight and obese ones, and the associations between PNPLA3 gene polymorphisms and NAFLD and between TM6SF2 gene polymorphisms and triglycerides level were stronger in lean than overweight and obese subjects. Therefore, genetic polymorphisms may have a greater effect on the risk of NAFLD and disease progression in non-obese/lean subjects, but inconsistent findings has been observed across different ethnic groups; in addition, the interaction between adiposity and genetic variants other than PNPLA3 gene polymorphisms on the development and progression of NAFLD have not been adequately evaluated in previous Asian studies. Further research is needed to address genetic variants in non-obese or lean patients with NAFLD.

Effective therapeutic strategies for NAFLD remain unclear, and lifestyle modification including physical activity is the cornerstone for the management of NAFLD. In a recent randomized controlled trial of 154 community patients with NAFLD, a 12-month lifestyle intervention program involving regular exercise was an independent factor associated with remission of NAFLD in non-obese patients. Indeed, half of non-obese patients achieved NAFLD remission with a 3–5% weight reduction; the same could only be achieved in obese patients with a 7–10% weight reduction. Another recent cohort study of 11,690 patients with NAFLD reported that the prevalence of significant liver fibrosis (fibrosis-4 index: 3.0%–1.0%; NAFLD fibrosis score: 2.4%–0.4%) and a high probability of atherosclerotic CVD (10.3%–6.3%) significantly decreased with increasing amounts of physical activity by quartile in lean patients with NAFLD. These results showed that lifestyle intervention could be effective in treating NAFLD in non-obese patients.

Currently, there are no effective approved pharmacologic treatments for NAFLD. Moreover, since most clinical trials related to drug development target patients with NAFLD and severe obesity, it is unclear whether these drugs can be applied equally to non-obese or lean patients with NAFLD. Vitamin E and pioglitazone were efficacious for biopsy-proven nonalcoholic steatohepatitis (NASH) in clinical practice; however, these agents should be used with caution in selected

**Abbreviations:**
BMI, body mass index; CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing-3; TM6SF2, transmembrane 6 superfamily member 2

patients due to the reported risk in both European and American guidelines. A recent placebo-controlled, randomized study of 50 lean patients with NAFLD who underwent lifestyle modification interventions reported that a symbiotic supplement consisting of seven bacterial strains resulted in a significantly greater reduction in liver stiffness (transient elastography: 9.36 kPa → 6.38 kPa in the symbiotic group and 7.92 kPa → 7.16 kPa in the placebo group), insulin resistance, and inflammatory markers including high-sensitivity C-reactive protein and nuclear factor-κB activity than placebo after a 28-week treatment period. Recently, randomized clinical trials have examined the efficacy of glucose-lowering agents for treating patients with biopsy-proven NASH, but most targeted obese patients with NAFLD. Among these studies, a phase-2b randomized placebo-controlled trial of 276 non-obese (overweight) or obese patients with biopsy-proven NASH reported that elafibranor (120 mg dose), a dual peroxisome proliferator-activated receptor-α/δ agonist, was significantly associated with a 2-point improvement in NAFLD activity score (48% elafibranor vs. 21% placebo) without worsening fibrosis (20% elafibranor vs. 11% placebo). However, large-scale clinical trials are needed to study the efficacy of glucose-lowering agents in non-obese or lean patients with NAFLD.

Non-obese or lean patients with NAFLD show clinical findings, such as insulin resistance or metabolic dysfunction, similar to those of obese patients with NAFLD; however, genetic polymorphisms and other factors may be responsible for NAFLD development in non-obese or lean individuals. Because time-dependent covariates such as changes in alcohol consumption or body weight are frequently encountered in patients with NAFLD, it is difficult to accurately evaluate the influence of time-dependent covariates on the prognosis of non-obese or lean patients with NAFLD. However, the general management and follow-up needs of non-obese or lean patients with NAFLD are similar to obese patients with NAFLD, and well-designed clinical studies for pharmacologic treatments on this population should be conducted in the future.

**Authors’ contribution**

All authors were responsible for the interpretation of data, the drafting, and critical revision of the manuscript for important intellectual content.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**


Non-obese or lean nonalcoholic fatty liver disease matters, but is it preventable or inevitable in light of its risk factors?

Heejoon Jang¹,² and Won Kim¹,²
¹Division of Gastroenterology and Hepatology, Department of Internal Medicine, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul; ²Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Keywords: Non-alcoholic fatty liver disease; Risk factors; Obesity; Metabolic diseases; Epigenomics

To date, a majority of studies have focused on obese nonalcoholic fatty liver disease (NAFLD), which is mainly responsible for the Western NAFLD population. Nevertheless, a substantial number of individuals with NAFLD in the Asia–Pacific region is not obese. Approximately 40% of the global NAFLD population is not obese and one-fifth is lean.¹ Furthermore, non-obese or lean NAFLD and obese NAFLD populations show comparable histological severity and long-term hepatic or extrahepatic outcomes.² In this regard, recent studies have been conducted to identify relevant risk factors beyond obesity for lean or non-obese NAFLD.

In this issue of Clinical and Molecular Hepatology, Ko et al.³ address the various risk factors that contribute to the development and progression of NAFLD, focusing on the lean or non-obese phenotype. Recent evidence indicates that central obesity rather than obesity per se driven by body mass index is associated with fibrosis severity in non-obese NAFLD.² Specifically, visceral adiposity plays a more important role in the pathogenesis of NAFLD than does subcutaneous adiposity in terms of changes in NAFLD status.⁴ Sarcopenia is bidirectionally associated with NAFLD, although myosteatosis rather than sarcopenia plays a more important role in the progression of early-stage NAFLD.⁵ In addition, other medical conditions, such as type 2 diabetes and genetic polymorphisms, may also affect the development and progression of NAFLD in a lean population. A high caloric or fructose diet is considered a modifiable risk factor for NAFLD regardless of obesity status. Given that NAFLD affects more than one-quarter of the global population and has become a threat to global health, researchers are encouraged to explore modifiable risk factors to establish an appropriate treatment for NAFLD.

Non-obese or lean NAFLD is associated with a subset of risk factors that cannot be modified: age, sex, ethnicity, and genetics. The prevalence of NAFLD increases with age. Indeed, the prevalence of NAFLD was approximately 42.2% higher...
among people older than 50 years compared with those younger. Nonalcoholic fatty liver disease is usually more prevalent in men than in women. According to a meta-analysis of studies published between 2016 and 2021, 44.5% of men and 31.8% of women had NAFLD. However, postmenopausal women had more severe NAFLD than men of similar age. The prevalence of NAFLD varies across ethnicities. In the United States, Hispanics have the highest prevalence of NAFLD, whereas African Americans have the lowest despite their higher rate of obesity. Genetic factors also contribute to the development and progression of NAFLD. For example, genetic variants such as PNPLA3 and TM6SF2 have been shown to be associated with the histological severity of NAFLD. A dose-dependent association has been observed between the G allele in PNPLA3 rs738409 and fibrosis progression. Moreover, a higher level of AGXT2 expression regulated by rs2291702 has a protective effect against liver fibrosis in patients with NAFLD.

Non-obese or lean NAFLD is also associated with several risk factors that can be modified: metabolic health, circulating metabolites, muscle mass and quality, the gut microbiome, and diet. Although obesity contributes to the prevalence and severity of overall NAFLD, metabolic health status has a greater effect on histological severity in non-obese compared to obese NAFLD. Altered circulating saturated sphingomyelin level is associated with the histological severity of non-obese NAFLD. Dysregulation of circulating unconjugated primary bile acids is also associated with nonalcoholic steatohepatitis (NASH) independent of obesity and diabetes. Crosstalk between the liver and muscle has a significant effect on the pathogenesis of NAFLD. Lower muscle strength and lower muscle mass are significantly associated with advanced fibrosis in the NAFLD population. Moreover, mortality risk was two-fold higher in individuals with both NAFLD and sarcopenia than in those with neither. Muscle quality (i.e., severe myosteatosis) rather than muscle quantity (i.e., lower muscle mass) is also significantly associated with NASH and fibrosis progression in early-stage NAFLD. Nonalcoholic fatty liver disease is substantially influenced by gut dysbiosis. Only individuals with non-obese NAFLD exhibit significant alterations in the diversity and composition of the gut microbiome and in stool metabolites along with increasing fibrosis severity. This suggests that gut-directed pharmabiotics may be a promising preventive and therapeutic strategy against non-obese NAFLD.

Growing evidence suggests that several epigenetic factors are also linked to NAFLD. An accompanying alteration in gene expression is associated with liver injury and NASH. Based on an assay for transposase-accessible chromatin with sequencing, substantial differences were noted in chromatin accessibility in the genomes of patients with non-NAFLD, nonalcoholic fatty liver, or fibrotic NASH. In addition, the length of telomeres in liver tissue cells shortens as fibrosis stage advances in patients with biopsy-proven NAFLD, even after adjustment for age.

Non-obese or lean NAFLD is a multi-factorial and complex condition that is influenced by both modifiable and non-modifiable risk factors. Although several risk factors, such as age and genetics, cannot be changed, many other risk factors exist that can be altered through lifestyle modifications and therapeutic interventions. In the near future, gene-based precision medicine, including anti-sense oligonucleotides and RNA interference, may alter the effect of genes on the development and progression of NAFLD, rendering genetics modifiable. Further studies on genetics and epigenetics will contribute to understanding the pathogenesis of non-obese or lean NAFLD, allowing us to develop potential therapeutic options for this disease. Better knowledge of modifiable risk factors would also assist in preventing or retarding the progression of NAFLD.

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

Acknowledgments
This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (2021R1A2C2005820 and 2021M3A9E4021818), Research of Korea Centers for Disease Control and Prevention (2022-ER0902-01).

Abbreviations:
NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis

http://www.e-cmh.org
Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

Implications of comorbidities in nonalcoholic fatty liver disease

Sherlot Juan Song1,2 and Vincent Wai-Sun Wong1,2

1Medical Data Analytics Centre, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong; 2State Key Laboratory of Digestive Disease, Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China

Keywords: Non-alcoholic fatty liver disease; Diabetes mellitus; Obesity; Sleep apnea

Nonalcoholic fatty liver disease (NAFLD) affects around 30% of the global adult population and is becoming a major cause of cirrhosis, hepatic decompensation and hepatocellular carcinoma. Ever since the initial characterization of NAFLD, we have known that obesity and insulin resistance are the main drivers of the disease. Numerous studies have confirmed a strong association between NAFLD and essentially all obesity-related metabolic disorders. Moreover, although as hepatologists we should pay attention to liver-related morbidity and mortality, it is clear that most NAFLD patients still die from cardiovascular disease (CVD) and extrahepatic cancers, with the exception of patients with cirrhosis in whom liver disease is the primary cause of death.

In this issue, a narrative review by Manikat and Nguyen explores the potential impact of NAFLD on the cardiovascular, renal, respiratory, endocrine systems as well as its association with non-hepatic cancer, infection and patient-reported outcomes. Some of these comorbidities can be alarming given that CVD and extrahepatic malignancies are the main culprits for mortality of NAFLD patients. The link between NAFLD and extrahepatic malignancies is worth our great attention, considering that a recent meta-analysis suggests the link is independent of potential confounders including obesity and diabetes.

However, the mechanisms underlying most of these associations remain unclear. As stated in the review and a previous study, the close relationship between NAFLD and metabolic syndrome contributes to the predisposition to CVD. Although shared metabolic dysfunction among these comorbidities enables clinicians to implement treatment strategies (i.e., life simple 7 guidelines) that may benefit both conditions, untraditional risk factors (i.e., fatigue and its driver sleep disturbances) may also affect NAFLD mortality through unknown pathways. Therefore, to clarify the nature of their associations, or whether and how NAFLD independently confers additional risk for other comorbidities may contribute to a more systematic management approach for NAFLD.
IS THE ASSOCIATION BETWEEN NAFLD AND ITS COMORBIDITIES CAUSAL?

While the association between NAFLD and its comorbidities is firmly established, one interesting question is whether the relationship is causal. In clinical medicine, randomized controlled trials are the gold standard to establish causality. However, it is impossible (e.g., NAFLD) or unethical (e.g., smoking) to assign some exposures to people. In 1965, Sir Hill published his nine-point criteria to support potential causality between environmental exposures and diseases. Since then, the criteria have often been used in epidemiological studies to infer causality. As illustrated in the Table 1, current studies have shown rather consistent correlation between NAFLD and comorbidities with a wide range of association strength depending on the condition. The bidirectional association (NAFLD preceding a comorbidity and vice versa) may be interpreted as both conditions contributing to the development of each other, or it may also mean the relationship is not causal. In some conditions (e.g., diabetes, chronic kidney disease, colorectal neoplasm), a dose-response relationship exists (i.e., the association with the comorbidity is stronger in patients with more severe liver disease). Finally, mechanistic studies to explain how NAFLD causes the extrahaepatic conditions are largely lacking.

Another caveat looms large in the clinical association studies. Some studies defined NAFLD and fibrosis using formulae, which typically comprise liver enzymes plus metabolic risk factors. For example, fatty liver index, one of the most commonly used scores to define NAFLD in large registry studies, includes waist circumference, body mass index and triglycerides. As such, it would be impossible to fully dissect the true effect of NAFLD from adiposity and metabolic dysfunction.

SOME EXAMPLES ON POTENTIAL CAUSAL RELATIONSHIPS

Type 2 diabetes (T2DM)

In a systematic review and meta-analysis of 80 studies, the global prevalence of NAFLD is estimated at 56% among patients with T2DM. Over 10% of patients with T2DM may have liver fibrosis progression in 3 years. NAFLD also increases the risk of incident diabetes by 2-fold. Among patients with NAFLD and T2DM, age is a major determinant of adverse liver-related outcomes, whereas the use of aspirin, statins and some classes of anti-diabetic drugs (e.g., metformin and pioglitazone) has been shown to reduce the risk of hepatocellular carcinoma and cirrhotic complications.

A number of mechanisms have been suggested to explain the increased risk of T2DM in patients with NAFLD. These include common dietary factors (e.g., fructose and saturated fat), gut microbiota dysbiosis (e.g., short-chain fatty acids produced by gut bacteria, such as butyrate, can modulate insulin sensitivity), increased gut permeability, adipose tissue dysfunction, changes in de novo ceramide synthesis, and increased hepatic glucose production (i.e., manifestation of hepatic insulin resistance). A recent prospective study reported the prevalence of NAFLD among patients with T2DM to be 65.3%, and patients who developed NAFLD were more likely to have obesity and the metabolic syndrome. Interestingly, NAFLD was also a risk factor in the development of T2DM in some prospective cohort studies, and changes in steatosis status correlated with the incidence of T2DM through 15-year long-term follow-up, adding the robustness of association between two diseases. However, with many metabolic confounders, a causative relationship remains unclarified.

Obstructive sleep apnea (OSA)

There is growing evidence that OSA severity manifested as intermittent hypoxia is dose-dependently associated with the development and progression of NAFLD. Various experimental evidence shows that intermittent hypoxia leads to glucose and lipid dysregulation, and hepatic inflammation, oxidative stress, and fibrosis, all of which are critical factors in NAFLD development. OSA may increase the risk of NAFLD through its independent impact on insulin resistance, as supported by both clinical and animal studies. Of note, the association between severe OSA and liver fibrosis evaluated by

Abbreviations:
NAFLD, nonalcoholic fatty liver disease; CVD, cardiovascular disease; OSA, obstructive sleep apnea; ALT, alanine aminotransferase; CAD, coronary artery disease; MR, Mendelian randomization; T2DM, type 2 diabetes
**Table 1.** Bradford Hill criteria for causation

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Do studies reporting the association between NAFLD and comorbidities fulfill the criteria?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of association</td>
<td>An odds ratio or relative risk of $\geq 2$ is generally regarded as a strong association. This has been seen for some but not all comorbidities.</td>
</tr>
<tr>
<td>Consistency</td>
<td>The literature on the positive association is largely consistent.</td>
</tr>
<tr>
<td>Specificity</td>
<td>It would be difficult if not impossible to establish specificity in this case. Obesity-related metabolic disorders tend to occur together. Even if a causal relationship exists, NAFLD would unlikely be the sole cause of the comorbidity.</td>
</tr>
<tr>
<td>Temporality</td>
<td>Bidirectional relationship is more commonly reported. For example, patients with NAFLD are more likely to develop incident type 2 diabetes over time, whereas patients with type 2 diabetes also have increased risk of incident NAFLD and liver fibrosis. Another issue is that the onset of NAFLD is often inaccurate because the disease is largely silent before the development of liver-related complications.</td>
</tr>
<tr>
<td>Biological gradient</td>
<td>The severity of NAFLD (i.e., presence of NASH or the degree of liver fibrosis) has been shown to correlate with the risk of some comorbidities (e.g., type 2 diabetes, chronic kidney disease, colorectal neoplasm) in a dose-dependent manner.</td>
</tr>
<tr>
<td>Plausibility</td>
<td>Because of the well-established crosstalk between the liver and other organs (e.g., gastrointestinal tract and adipose tissue) and that adipose tissue is a major source of lipids to the liver, it is reasonable to consider obesity (or visceral adiposity) as a cause of NAFLD. The other way round (NAFLD causing the comorbidities), however, is more difficult to discern apart from the fact that NAFLD is a state of marked insulin resistance.</td>
</tr>
<tr>
<td>Coherence</td>
<td>There has not been serious conflict in the data interpretation with what is known about NAFLD and its comorbidities.</td>
</tr>
<tr>
<td>Experiment</td>
<td>The mechanisms underlying the association between NAFLD and comorbidities are not established in most cases. However, mechanistic studies exist in some conditions (e.g., hypoxia from obstructive sleep apnea causing NAFLD; NAFLD being a state of hepatic insulin resistance).</td>
</tr>
<tr>
<td>Analogy</td>
<td>There is no good analogy to suggest a similar condition may cause a similar disease.</td>
</tr>
</tbody>
</table>

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.
liver stiffness measurement remains after multivariate adjustment for metabolic factors including insulin resistance.20

Although alanine aminotransferase (ALT) and aspartate aminotransferase levels are lowered with the continuous positive airway pressure treatment,21 most related studies were limited by either small cohorts or short duration and failed to confirm its role in NAFLD progression.22 Further studies are needed to clarify the underlying mechanisms as well as treatment strategies for these two disorders.

Coronary artery disease

As a top killer of NAFLD patients, CVD, particularly coronary artery disease (CAD) among its spectrum, is widely discussed for its close association with poor NAFLD prognosis. A meta-analysis of six studies with 25,837 patients reported that NAFLD patients had a 2.2-fold higher risk of developing CAD than those without NAFLD.23 In a large cohort of asymptomatic individuals in South Korea, NAFLD was an independent risk indicator of subclinical coronary atherosclerosis with manifestation of having non-calcified coronary atherosclerotic plaques.24 These vulnerable plaques suggest high risk of unexpected adverse cardiovascular events.

The association between CAD and NAFLD is well-established but rather intricate with both traditional and non-traditional metabolic risk factors involved, such as abnormal glucose metabolism, insulin resistance, hyperadipocitinemian, and hyperuricemia.25 As mentioned, dysfunctional and ectopic lipid accumulation in NAFLD can be the damage-driver, causing lipoprotein abnormalities, initiation of inflammatory response and consequent development of atherosclerosis.26 In this context, pericardial accumulation of ectopic fat represents another central pathogenesis for cardiometabolic disorders.27 All these pathological hallmarks promote the development of CVD. However, the precise causative relationship is still under investigation.

MENDELIAN RANDOMIZATION STUDIES: A NEW TOOL TO ESTABLISH CAUSALITY?

A recent study contributes to inferring causality between NAFLD and CAD using an emerging method - Mendelian randomization study (MR).28 MR is based on the assumption that certain genetic variants affect outcomes of interest (i.e., CAD) only through the exposure of interest (i.e., NAFLD) and independent of other confounders. By using MR, they demonstrated the interlink between genetically predicted NAFLD (defined as chronically increased ALT levels, imaging--based, or biopsy--confirmed NAFLD) and CAD after excluding generic variants involved in impaired very-low density-lipoprotein secretion. However, another MR study focusing on the association of NAFLD with CVD events observed no significant relationship between NAFLD and CAD, heart failure, or stroke.29 Among different CVD events, only arterial stiffness was found to be causally associated with NAFLD. Wu et al.30 also found no causal effect between NAFLD and stroke using the MR method, although a causal relationship might exist for ischemic stroke, large artery atherosclerosis and small vessel occlusion. This may be mediated by deranged adipokine profile and abnormal high-density lipoprotein cholesterol level in NAFLD patients. Further studies investigating the relationship between NAFLD and CVD are warranted.

Xie et al.31 also sheds light on the complex relationships between NAFLD and its comorbidities using the MR method. Focusing on a number of modifiable risk factors, the widely reported associations between genetically predicted T2DM, hypertension and hypothyroidism with NAFLD were further confirmed. Notably, no significant association existed between hypothyroidism and NAFLD after adjusting for genetically predicted body mass index, suggesting that this relationship could be confounded by adiposity. Other causative risk factors for NAFLD including alcohol frequency, elevated serum levels of liver enzymes, obesity were also indicated in their integrated databases, which provides references for the public health intervention and the routine management for NAFLD. However, the database includes only European individuals, thus the generalizability of this study remains to be verified. In addition, pleiotropy is still a big challenge for all MR studies to overcome.

MANAGEMENT IMPLICATIONS

In summary, the excellent review by Manikat and Nguyen3 is a timely reminder of the strong association between NAFLD and various comorbidities. While causality of the association remains a matter of debate, clinicians should be aware of the important comorbid conditions and arrange assessment and treatment as appropriate. Accordingly, the cur-
rent Asia-Pacific guidelines recommend routine assessment for adiposity, blood pressure, glucose and lipids in patients with NAFLD. Accumulating data also support measurement of kidney function, and if symptomatic, assessment for obstructive sleep apnea and CVD. In addition, colorectal cancer and breast cancer are more common in patients with NAFLD, and screening has been shown to detect early cancers and lower mortality. Future studies should define whether a diagnosis of NAFLD should prompt earlier and more frequent screening for cardiometabolic conditions and related cancers.

Authors’ contribution
Both authors contributed to the writing plan, literature review, and manuscript preparation. They approved the final version of this article.

Acknowledgements
This work was supported by a direct grant from The Chinese University of Hong Kong (project reference 2021.027).

Conflicts of Interest
Vincent Wong has served as an advisor or consultant for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Intercept, Inventiva, Novo Nordisk, Pfizer, Sagimet Biosciences, and TARGET PharmaSolutions; and a speaker for Abbott, AbbVie, Gilead Sciences, and Novo Nordisk. He has received a research grant from Gilead Sciences, and is a co-founder of Illuminatio Medical Technology Limited. Sherlot Song reports no conflict of interest.

REFERENCES
Sherlot Juan Song, et al.  
Comorbidities of NAFLD

http://www.e-cmh.org
https://doi.org/10.3350/cmh.2023.0066


Screening strategies for non-alcoholic fatty liver disease: a holistic approach is needed

Philipp Kasper¹, Münevver Demir², and Hans-Michael Steffen¹,3

¹University of Cologne, Faculty of Medicine, and University Hospital Cologne, Department of Gastroenterology and Hepatology, Cologne; ²Department of Hepatology and Gastroenterology, Campus Virchow Clinic, Charité University Medicine, Berlin; ³University of Cologne, Faculty of Medicine, and University Hospital Cologne, Hypertension Center, Cologne, Germany

Keywords: Liver; NAFLD; Cardiovascular disease; NASH; HCC

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide, currently affecting around 32.4% of the adult population.¹ In addition to the adult population, there has also been a significant increase in the incidence and prevalence of NAFLD among children and adolescents over the last decade, which was further aggravated by the COVID-19 pandemic.²³ The prevalence of NAFLD in children is estimated to range from 5–10% in the general population, rising up to 30% in young individuals with obesity.³

Therefore, NAFLD represents a growing public health problem across all age groups of the population, causing a tremendous clinical and socio-economic burden.⁴⁵

NAFLD encompasses a spectrum of liver disorders ranging from simple hepatocellular steatosis (non-alcoholic fatty liver, NAFL) to more progressive steatohepatitis (NASH) with varying degrees of fibrosis, and ultimately cirrhosis.⁵ NAFLD is considered as the hepatic component of the metabolic syndrome and is associated with an increased risk of the development of various liver-associated and cardiometabolic complications.⁶⁷

Individuals with NAFLD have an approximately two-fold higher risk of developing type 2 diabetes (T2DM) and NAFLD is associated with an increased long-term risk of fatal or non-fatal cardiovascular events.⁷⁸

Alongside the growing epidemics of obesity and metabolic syndrome, the NAFLD disease burden is expected to increase during coming years.⁹

Global estimates currently suggest that the prevalence of NAFLD will continue to increase significantly through 2030, with a concomitant increase in the proportion of patients with advanced disease stages (e.g., advanced liver fibrosis), who are at the highest mortality risk.⁹ In the Asian-Pacific region, cases of NAFLD will increase by up to 20% until 2030 and NAFLD-related mortality is projected to increase by more than 65%.¹⁰

Due to the alarming surge in prevalence and incidence of NAFLD in various groups of the population and the associated major health risks, particularly in patients with progressive disease, it is time to consider screening programs for NAFLD.
In their comprehensive review, Zhang and colleagues address this important issue and provide an up-to-date overview of screening strategies for NAFLD.

The authors discuss thoroughly general requirements for screening programs and their cost-effectiveness and describe current international guideline recommendations. Further, the authors provide a detailed overview of different screening modalities including their advantages and disadvantages (e.g., serum-based methods, ultrasonography, transient elastography, magnetic resonance imaging), and evaluate suitability of different techniques for screening purposes in clinical practice. The authors emphasize that non-invasive screening measures are particularly relevant in specific high-risk groups, including patients with T2DM, metabolic syndrome or persistently elevated liver enzymes, whereas screening of the total population is generally not advisable and not cost-effective.

Finally, the authors present a two-step-assessment scheme with serum-based fibrosis-4 index (FIB-4) or NAFLD fibrosis score (NFS) followed by an imaging test, i.e. vibration-controlled transient elastography, as an option to stratify the risk of liver-related complications. Zhang et al. provide a comprehensive and relevant review for everyday clinical practice. Non-invasive testing strategies using easily available laboratory data for detecting advanced NAFLD disease stages in distinctive risk groups are crucial, since these patients have a high mortality risk and frequently suffer from liver-related complications.

However, most NAFLD patients worldwide are not in advanced disease stages when NAFLD is diagnosed and do not die primarily from liver-related events.

The majority of patients suffer from early stages of NAFLD and cardiovascular diseases (CVD) are the leading cause of death in patients with NAFLD. This can partly be explained by the fact that patients with NAFLD often exhibit multiple cardiovascular risk factors such as atherogenic dyslipidemia, T2DM or arterial hypertension, which are often neither recognized nor adequately controlled.

As there is growing evidence for NAFLD as a ‘multisystem’ disease, concomitant risk factors such as obesity and additional cardiometabolic disorders should also be considered in the context of planned screening measures.

Therefore, in addition to the identification of patients with advanced stages of liver fibrosis, particular attention should be paid to detection, treatment and adequate control of T2DM, hypertension, and atherogenic dyslipidemia, which are interrelated with NAFLD severity and key determinants of the individual cardiovascular risk.

Recent data clearly show, that with the increase in the proportion of adequately treated cardio-metabolic comorbidities, the rate of adverse CVD events as well as the CVD mortality dramatically decrease in patients with NAFLD. Thus, optimal screening and management of cardiovascular comorbidities are imperative in this high-risk population and current liver-centered approaches need to be complemented by effective screening, diagnostic, and treatment strategies for cardiovascular comorbidities. Screening measures for CVD should include: i) evaluation of family history for CVD, smoking status, physical activity and alcohol consumption, ii) laboratory analysis of lipid levels (e.g., total cholesterol, low-density lipoprotein, triglycerides), glucose metabolism and glycemic control (e.g., fasting glucose, glycated hemoglobin) and renal function (e.g., glomerular filtration rate), iv) blood pressure measurement (preferred by office and 24-hour ambulatory blood pressure measurement in case of high normal office blood pressure or grade 1 hypertension to exclude masked or white coat hypertension), v) estimation of CVD risk by specific risk assessment models and scoring systems. Interestingly, FIB-4 as well as NFS have recently been shown to be predictive of all-cause and cardiovascular mortality.

Besides the important question “How should screening be performed?”, the question “Who should be screened? “ is also essential.

Screening programs should not only be carried out in adults, but also in children and adolescents.

Due to the ongoing global childhood obesity epidemic, it has become apparent, that NAFLD is also a major health problem in younger people with overweight or obesity and adversely affects the health of those individuals in the long-term.

Recent studies have clearly shown that children and young

---

**Abbreviations:**
NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes; FIB-4, fibrosis-4; NFS, NAFLD fibrosis score; CVD, cardiovascular diseases
adults with biopsy-proven NAFLD had significantly higher rates of incident major adverse cardiovascular events later in life, including ischemic heart disease and congestive heart failure, when compared with matched controls without NAFLD. In addition, children with NAFLD are at potentially higher risk for developing T2DM and dyslipidemia.

As metabolically unhealthy children will grow up into cardiometabolic ill adults when they get older, NAFLD screening measures should already be implemented in the pediatric population (e.g., school entry medical examination programs) to mitigate the incoming wave of young multimorbid NAFLD patients.

Although progression to end-stage liver disease generally takes decades, without selective measures there will be a substantial burden of liver disease and other cardiometabolic diseases in 50-year-olds in the near future.

In the debate on “who should be screened”, it is also important to establish specific cut-off values of the proposed non-invasive scoring systems.

Liver biopsy can securely be replaced only with a stepwise combination of noninvasive tests, otherwise the assessment of risk due to advanced fibrosis may be misleading in a clinically meaningful proportion of patients. It is along that line that we believe a low positive predictive value and high false-positive rate can be tolerated. Hence, even lower FIB-4 cut-off values, e.g., 1.0, may be more appropriate for screening purposes as proposed by Shah and colleagues. Using the traditional FIB-4 cut-off of 1.3, the specificity for advanced fibrosis has been found to be unacceptably low in patients aged ≥65 years, and Zhang et al. have adopted the threshold of 2.0 for use in these patients as has been previously proposed.

When focusing upon the question “Who should perform screening?”, the task will now be to prime frontline primary care physicians for the projected NAFLD burden, who predominantly care for these patients in everyday clinical practice.

In addition to medical education programs, which must be established in order to train the practitioners in the detection of NAFLD patients, school education campaigns must also be created and superordinate structures (e.g., insurance companies, healthcare authorities, policy maker) must support the financing and implementation of such health programs. NAFLD screening requires multidisciplinary care and can only succeed with a multistakeholder approach. If the framework conditions are not optimal, every planned screening measure will fail.

In concrete terms, all users of screening measures must be provided with uniform algorithmic approaches including practical recommendations which can be used in everyday clinical practice. Here, it should be noted that primary non-invasive risk assessment tools and scoring systems (e.g., fatty liver index, FIB-4 index) can actually be used on a broad scale in the outpatient sector, since instrument-based procedures such as elastography are not broadly available in primary care. A provision of health services must be created where frontline primary care physicians can easily and effectively identify patients with NAFLD by using simple tools with further diagnostic evaluation of disease severity provided by specialized facilities.

Ultimately, it is also important that screening measures do not have to take place just once, but at regular intervals, for example as part of health insurance-based preventive programs.

In conclusion, given the growing global NAFLD burden, holistic screening approaches for NAFLD are needed, including a comprehensive assessment of important (cardiovascular) diseases beyond the degree of liver damage. Furthermore, successful implementation of screening measures requires an improvement in awareness of the health risks of NAFLD, which can only be achieved through the concerted action of different healthcare stakeholders.

Authors’ contribution
PK wrote the manuscript. MD and HMS revised the manuscript critically for important intellectual content.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
3. Yu EL, Schwimmer JB. Epidemiology of pediatric nonalcoholic


Non-invasive imaging biomarkers for liver steatosis in non-alcoholic fatty liver disease: present and future

Lynna Alnimer¹ and Mazen Noureddin²

¹Division of Gastroenterology, Ascension Providence Hospital, Michigan State University/College of Human Medicine, Southfield, MI; ²Houston Research Institute, Houston, TX, USA

Keywords: Steatosis; Fibrosis; Noninvasive; Imaging; NAFLD

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in the United States.¹ It is not only associated with cirrhosis but is also considered a significant risk factor for cardiovascular disease and other complications related to the metabolic syndrome.² NAFLD is considered an umbrella term for a group of diseases; the spectrum starts with nonalcoholic fatty liver (NAFL), which is defined as liver fat content of more than 5% of the hepatocyte and is characterized histologically by macrovesicular hepatic steatosis. NAFL can progress into non-alcoholic steatohepatitis (NASH) which is characterized by the presence of inflammation and cellular injury, specifically ballooning, with or without fibrosis. However, both NAFL and NASH are associated with an increased risk of fibrosis and identifying it at an early stage is key. NAFL progresses to NASH in up to 30% of cases, leading to significant liver fibrosis with detrimental consequences.² Although liver biopsy remains the gold standard to diagnose NASH and liver steatosis, it is associated with risks and challenges. In addition, the rise of noninvasive testing (NITs) is found to be easier to perform, cost-effective, and less invasive.²

Given the prevalence of NAFLD worldwide, it is not feasible to perform liver biopsies on all patients with the suspected disease. There are several limitations of liver biopsy including sampling error, inter- and intra-observer variability, risks, and complications. Since only around 1/50,000 of the whole liver tissue is sampled during one biopsy, this by itself raises the concern of sampling error.³ Hepatocyte ballooning is a histological key feature differentiating steatosis (NAFL) from NASH yet expert liver pathologists disagree in many instances on the presence or absence of ballooning.³ Moreover, every procedure is associated with risks, and the incidence of serious complications and mortality has been reported to be 0.3–0.57% and 0.01% respectively.⁵ As mentioned by Nogami et al.⁶, the importance of assessing the degree of fibrosis rather
than diagnosing NAFL or NASH or evaluating liver steatosis is key. Nogami et al. also mentioned the importance of appropriately evaluating liver steatosis as studies have shown higher risk of mortality from extrahepatic cancer, cardiovascular disease, cirrhosis and hepatocellular carcinoma with different liver steatosis levels. Nevertheless, NITs play a significant role nowadays in diagnosing and managing multiple aspects of NAFLD including identifying disease severity, monitoring response to therapy, and predicting outcomes.

**FIRST GENERATION TESTS**

There are different serum biomarkers and composite scores used to evaluate hepatic fibrosis such as the Enhanced Liver Fibrosis (ELF) test and Fibrosis-4 (FIB-4), but the scope of this editorial revolves around the role of imaging in liver steatosis. As mentioned in the recently published review article by Nogami et al., multiple tests exist to identify steatosis including abdominal ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). US is a simple and popular type of imaging to diagnose fatty liver. B mode findings such as bright liver, vascular blurring attenuation, and hepatorenal echo contrast indicate fatty liver however the sensitivity and specificity decrease when intra-hepatic steatosis is less than 30%. CT scans can identify fatty liver, yet they are costly, time-consuming, a relatively poor indicator to quantify steatosis, and are associated with inevitable radiation exposure. On CTs, fatty liver is usually diagnosed by comparing the liver fat content relative to that of the spleen. MRI is an excellent method to quantify fat content in the liver as the signals are obtained from protons belonging to water and fat molecules, and there is no risk of exposure compared to CT. However, it has not been used in general practice due to high costs.

Vibration-Controlled Transient Elastography (VCTE) (e.g., FibroScan) was discovered in 2003 and is used to obtain a liver stiffness measurement (LSM) that correlates with fibrosis. It is widely available and can be used as a point-of-care test. In 2010, the controlled attenuation parameter (CAP) was introduced to measure the degree of fat attenuation, allowing it to quantify liver steatosis. Initially, obesity was considered a limitation until the XL probe was introduced, which allows for deeper penetration to generate signals in patients with a higher body mass index (BMI). As mentioned by Nogami et al., CAP is essential in evaluating S ≥1, 2, and 3 however it has not been reported whether the measurement of liver steatosis is useful for long term follow up. On the other hand, changes in liver stiffness can be used to identify disease progression.

Newer MR-based modalities are used to quantify hepatic fat. MRI proton density fat fraction (MRI-PDFF) is a MR technique that accurately quantifies hepatic fat by decomposing the signals obtained from the liver into its fat and water components. It minimizes most confounding factors, including patient factors such as body mass index (BMI), sex, age, or etiology of liver disease, or other liver abnormalities such as iron overload. Although MR approaches are considered the gold standard NIT to detect steatosis, they are not used as frequently due to limited availability and high cost. Importantly, MRI-PDFF can be coupled with magnetic resonance elastography (MRE) which is more sensitive than VCTE in the detection of fibrosis stage ≥2 and is considered the most accurate noninvasive imaging-based test in fibrosis assessment in NAFLD.

**SECOND GENERATION TESTS**

Steatohepatitis remains the driver of the disease, and thus regulators have considered NASH patients with the histological NASH activity score of 4 and higher and fibrosis stage 2 and higher (also known as at-risk NASH) as the targeted group for pharmacological therapy, especially in NASH phase 3 registry studies. Combining serologic markers with imaging is an improved way to assess at-risk NASH patients and has been studied recently. A newer predictive score combines LSM, CAP, and aspartate aminotransferase (AST) to-

---

**Abbreviations:**

NAFLD, nonalcoholic fatty liver disease; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NITs, Noninvasive testing; ELF test, Enhanced Liver Fibrosis (ELF) test; FIB-4, Fibrosis-4; US, abdominal ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; VCTE, Vibration-Controlled Transient Elastography; LSM, liver stiffness measurement; CAP, controlled attenuation parameter; BMI, body mass index
gether, known as FAST (FibroScan-AST), which is an efficient way to identify these individuals and minimize unnecessary liver biopsies. In a prospective multicenter study of 350 patients, the FAST score was internally and externally validated with a cutoff of 0.35 and 0.67 for ≥0.90 sensitivity and specificity, respectively, in the derivation cohort. Moreover, Agile 3+ and 4 are other non-invasive scores based on VCTE that accurately identify fibrosis (≥F3) and F4 (cirrhosis), respectively, but also predict adverse outcomes such as major adverse liver outcomes (MALO), hepatocellular carcinoma (HCC), the requirement for liver transplant (LT), and death. Last but not least, the MAST score (MRI-AST) is an MRI serum-based score that, by far, outperforms previous scores (FAST and FIB-4) in identifying at-risk NASH patients. The MEFIB score has also shown an ability to predict at-risk NASH patients and MALO. Nevertheless, the dichotomous nature of the test gives less flexibility to its use in comparison to MAST and FAST.

In terms of correlation between NITs and MALO, a study by Younossi et al. has shown that baseline FIB-4, NFS, ELF, and VCTE correlated with clinical liver outcomes. Boursier et al. have shown significant increases in patients’ risk for MALO with “FIB4 ≥1.30 then VCTE 8.0-12.0 kPa” (aHR 3.8; 95% CI 1.3–10.9) and even more for those with “FIB4 ≥1.30 then VCTE >12.0 kPa” (aHR 12.4; 95% CI 5.1–30.2). Two studies have shown that increases in MRE stiffness correlate with MALO and that a cutoff of 6.48 is a threshold of decompensation. In another study with six international cohorts, MRE was shown to be associated with liver outcomes; the MEFIB (a combination of MRE and FIB-4) had an excellent negative predictive value for hepatic decompensation. MAST has also shown a correlation with clinical liver events with c-Statistic of >0.92.

CONCLUSION
NAFLD is a progressive liver disease that can lead to cirrhosis. Its worldwide prevalence is high and continues to rise. In clinical practice, NITs are being used more frequently to identify steatosis, fibrosis, and high-risk NASH instead of liver biopsy, which is invasive, expensive, and associated with risks. Quantifying liver fat content is important however identifying “at risk NASH” is more essential. The future of NASH diagnosis and management is heading towards non-invasive methods, as there is robust evidence that NITs can assess disease severity and predict liver-related events. Ongoing studies are being conducted to support the use of NITs in monitoring responses to available treatments.

Authors’ contribution
Lynna Alnimer drafted the manuscript. Mazen Noureddin revised and finalized the manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Non-invasive biomarkers of liver fibrosis in non-alcoholic fatty liver disease

Maamon Basheer¹, Mohamed Naffaa², and Nimer Assy¹²³

¹Internal Medicine Department, ²Rheumatology Unit, Galilee Medical Center, Nahariya; ³Azrieli Faculty of Medicine in the Galilee, Bar-Ilan, University, Safed, Israel

Keywords: Fatty liver; Non alcoholic steato-hepatitis; Liver fibrosis; Non invasive markers

The search for reliable and non-invasive biomarkers of liver fibrosis has been a focus of intense medical research, with the aim of improving patient outcomes through early diagnosis and effective treatment. The purpose of this editorial is to raise awareness about the importance of non-invasive biomarkers for liver fibrosis and the impact of fibrosis on overall well-being.

Various predictive tests have been developed as non-invasive tests alternative to either imaging or liver biopsy.¹³ The Fibrosis Risk Stratification includes three levels: Low risk if (fibrosis-4 [FIB-4]: <1.30, liver stiffness measurement [LSM] <8 kPa, enhanced liver fibrosis [ELF] <7.7); Indeterminate risk if (FIB-4: 1.30–2.67, LSM 8–12 kPa, ELF 7.7–9.8) and high risk if (FIB-4: >2.67, LSM >12 kPa, ELF >9.8). However, these tests have often not met quality metrics for diagnostic tests, leaving the clinician with a fair degree of uncertainty.⁴⁻⁸

In this issue of the Clinical and Molecular Hepatology, Reinson et al.⁹ clearly showed that there are several biomarkers that have been studied for their ability to identify individuals with F2-F3 degree of fibrosis in the liver. These markers includes FibroTest, NAFLD fibrosis score, Fibro Scan, acoustic radiation force impulse, Aspartate aminotransferase-to-Platelet Ratio Index (APRI), magnetic resonance elastography (MRE), FibroScan-AST (FAST) score and ELF tests.⁵ It is important to note that these biomarkers are not perfect and their performance may vary depending on age, body mass index and the underlying cause of fibrosis. Therefore, it is important to use them in combination with other diagnostic tools, such as clinical examination and imaging exams, to make a definitive decision.

Machine learning algorithms have been studied as a future potential alternative for identifying individuals with F2-F3 fibrosis or higher; But their performance ability needs to be confirmed.¹⁰⁻¹¹

Noninvasive serum biomarkers have been studied for their utility in predicting liver-related outcomes.¹¹ Serial measurement of specific biomarkers could be used in order to monitor liver disease progression and response to treatment.¹¹ Some examples include tracking fibrosis by using FibroTest and APRI, monitoring cirrhosis by prothrombin time, and monitoring liver cancer by α-fetoprotein. Increase of 20% in
vibration controlled transient elastography (≥16.6 kPa) predicts progression to cirrhosis and ≥30.7 kPa predicts decompensation. Increase of 15% in MRE is associated with fibrosis progression and increase of 19% in MRE associated with poor outcomes. In viral hepatitis, measuring viral load, liver enzymes and FibroTest or FIB-4 at different intervals can help to assess the effectiveness of antiviral therapy. It is also important to consider that some biomarkers may not significantly change even with an effective treatment, or may take longer time to show improvement.

Non-invasive biomarkers can be also useful tools in drug trials for non-alcoholic steatohepatitis (NASH). These tools could help in monitoring progression and regression of liver fibrosis. However, it is important to use them in conjunction with other diagnostic tools and to have a clear understanding of their limitations and potential biases. Higher baseline, greater change in ELF (>9.76) is associated with an increased risk of progression to cirrhosis. ELF greater than 11.3 predicts liver-related clinical events.

Liver stiffness measurement by transient elastography, platelet counts and spleen stiffness could be also used for assessment of the degree of portal hypertension and the extent of liver fibrosis. A level of transient elastography above 15 kPa and platelet counts below 150k indicate significant portal hypertension.

How best can I Identify who needs to be treated without a liver biopsy?

There are three scores to answer that:

1. The FAST score (FibroScan AST) provides an efficient way to non-invasively identify patients at risk of progressive NASH for clinical trials or treatments, and thereby reduce unnecessary liver biopsy in patients unlikely to have significant disease. Performance of FAST score is good with AUC 0.71, positive predictive value (PPV) 33-85% and negative predictive value (NPV) 73-100%.

2. The MAST score (MRI-PDFF-AST) outperforms previous scores with AUC 0.93; In the validation cohorts, the 90% specificity cut-off of 0.242 corresponded to a sensitivity of 75%, PPV of 50% and NPV of 97%, whereas the 90% sensitivity cut-off of 0.165 corresponded to a specificity of 72%, PPV of 29%, and NPV of 98%.

3. Finally, the MRE combined with FIB-4: (FIB-4 [if ≥1.6]+MRE ≥3.3 kPa) score is superior to FAST in detecting patients “at risk” for NASH among patients with biopsy-proven NAFLD with AUC 0.88.

Future Perspective: metabolomics, lipid omics, and multiomics (gut microbiome) studies could help the clinicians in identify biomarkers associated with the pathophysiology of NAFLD and NASH. Integration of artificial intelligence and machine learning techniques to improve diagnostic accuracy and to develop personalized treatment plans for patients.

These biomarkers offer a more reliable, non-invasive, and cost-effective alternative to liver biopsy for diagnosing liver fibrosis. Combining several tests and scores, and creating charts for risk stratification and management, help the primary physician manage such patients and refer them to specialized centers.

Authors’ contribution

NIMER Assy wrote the manuscript. Maamon Basheer and Mohamed Naffah revised it.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

1. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA 2015;313:2263-2273.
3. Harrison SA, Ratziu V, Boursier J, Francque S, Bedossa P, Majd Z,

Abbreviations:

FIB-4, fibrosis-4; LSM, liver stiffness measurement; ELF, enhanced liver fibrosis; ARFI, acoustic radiation force impulse; NFS, NAFLD fibrosis score; APRI, aspartate aminotransferase-to-platelet ratio index; MRE, magnetic resonance elastography of liver; FAST score, FibroScan-AST score; VCTE, vibration controlled transient elastography; NASH, non-alcoholic steatohepatitis; PPV&NPV, positive and negative predictive values; NAFLD, non-alcoholic fatty liver disease; MAST score, The MRI-aspartate aminotransferase score; MEFIB, MRE combined with FIB-4


8. Alkhouri N, McCullough AJ. Noninvasive diagnosis of NASH and liver fibrosis within the spectrum of NAFLD. Gastroenterol Hepatol (N Y) 2012;8:661-668.


Non-invasive biomarkers for liver inflammation in non-alcoholic fatty liver disease: present and future

Kee-Huat Chuah and Wah-Kheong Chan

Gastroenterology and Hepatology Unit, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Keywords: Non-alcoholic steatohepatitis; Cytokeratin-18; MACK-3; Hepamet fibrosis score; Asia Pacific

We read with great interest the review article by Yip and colleagues.\(^1\) We could not agree more that the histological diagnosis of nonalcoholic steatohepatitis (NASH) can be substantially limited by sampling variability and observer variability. In one of the cited studies,\(^2\) there was only fair to moderate agreement between pathologists for the grading of lobular inflammation and hepatocyte ballooning and for the diagnosis of NASH. Importantly, there was an alarming disagreement rate between pathologists (i.e., in up to 23% of cases) for the diagnosis of NASH resolution without worsening of fibrosis (which is one of the key endpoints for NASH clinical trials). Furthermore, the semi-quantitative nature of grading and staging of the histological components may obscure changes following an intervention. We also agree with the authors that the liver biopsy procedure is invasive (with a small risk of serious complications, including mortality), and we have no reservation in stating that a liver biopsy is not feasible for routine clinical use for initiation of treatment and for monitoring of response in patients with nonalcoholic fatty liver disease (NAFLD), now or in the future.

While liver biopsy is a requirement for NASH clinical trials, it is also a major deterrent for patients to participate due to the fear of procedural risk. Furthermore, histology is a major cause of screen failures in clinical trials, which is partly attributable to its inherent limitations, as aforementioned. A pre-screening strategy using one or more non-invasive tests is often employed to reduce screen failure rates. However, as non-invasive tests were developed using histology as a reference standard, we are using tests that were constructed based on a problematic test to then select patients to be subjected to the problematic test for screening and enrollment into clinical trials. As much as the emphasis that has been placed on histological endpoints, they are but surrogate to clinical endpoints such as decompensation and liver-related mortality. There is an urgent need to demonstrate that non-invasive tests could act as a surrogate for these clinical endpoints and to determine the corresponding level and
the desired change for initiation of treatment and for monitoring of response, respectively.

In their review, Yip and colleagues pointed out that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) may be normal in patients with NASH and may even paradoxically decrease in patients with progressive fibrosis. We similarly observed that serum AST level has very poor negative predictive value for NASH. However, elevated serum AST level, especially when more than twice the upper limit of normal, has excellent positive predictive value for NASH. Moreover, a decrease in serum ALT level of 17 U/L or more has been found to be significantly associated with histological response. Importantly, these biomarkers are cheap and readily available. Although cytokeratin-18 (CK-18), an apoptotic marker, has limited role when used as a single test, combination of the test with other biomarkers have been found to correlate better with liver inflammation than routine tests. For example, we found that MACK-3 (combination of homeostatic model assessment (HOMA), AST and CK-18) has high diagnostic value for fibrotic NASH with an area under the receiver operating characteristic curve (AUROC) of 0.80. We also found that the diagnostic accuracy of MACK-3 for active NASH was the highest among the evaluated tests with AUROC, sensitivity and specificity of 0.81, 84.2% and 81.4%, respectively. Although HOMA, a marker of insulin resistance, is not routinely performed, its additional use in a fibrosis score with potentially improved performance may increase its role in the evaluation of patients with NAFLD.

Imaging studies, including ultrasound, vibration-controlled transient elastography (VCTE), computed tomography and magnetic resonance imaging (MRI) are useful as diagnostic modalities for NAFLD. However, only FibroScan-AST score, which utilizes VCTE and AST, and several MRI-based tests has been found to be promising in the measurement of liver inflammation. VCTE has the advantage of being non-invasive, reliable, easily performed and relatively affordable. In contrast, although the combinations of AST or fibrosis-4 index with MRI-proton density fat fraction, magnetic resonance (MR) elastography and/or iron-corrected mapping in MRI have high accuracy, the high cost and lack of availability may limit their use to only selected settings. Interestingly, Yip and colleagues also described the role of artificial intelligence in evaluating NASH. Supervised or unsupervised machine learning and deep learning models were able to improve the diagnostic accuracy of fibrotic NASH. For example, Fialoke et al. developed a machine learning model using large electronic health records from the United States and accurately predicted NASH based on longitudinal data of ALT, AST, platelet count, basic demographic information and diabetes status with AUROC of 0.83 to 0.88. Although machine learning models appear promising, more validation studies are needed before they can be applied to routine clinical use.

Due to the high prevalence of NAFLD but only a small yet significant proportion of patients have more severe liver disease, a simple assessment and referral pathway is necessary to ensure that patients with more severe liver disease are referred to specialist for further management. On the other hand, patients who are unlikely to have severe liver disease should remain in primary care, where they are best managed. An example of such assessment is the use of serum ALT and/or AST level among patients with type 2 diabetes mellitus, who are at higher risk of more severe liver disease, to identify patients who may have NASH. As serum ALT and AST level may be normal in patients with NASH, simultaneous assessment of liver fibrosis (e.g., with fibrosis-4 index, followed by liver stiffness measurement for patients with elevated fibrosis-4 index) will complement the evaluation and can serve as a safety net to identify patients with more severe liver disease but normal serum ALT and/or AST level. Another example is the use of a scoring system based on readily available parameters, for example, the Asia Pacific NASH Risk Score, which uses body mass index, diabetes mellitus, dyslipidemia, ALT and AST level. A score of 4 to 6 is considered as high-risk for NASH with NASH seen in 80% to 82.7% of patients. Until a more reliable and cost-effective screening or diagnostic test for liver inflammation becomes available, these simple and readily available tests may serve as part of the strategy to manage patients with NAFLD.

Abbreviations:
NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK-18, cytokeratin-18; HOMA, homeostatic model assessment; AUROC, area under the receiver operating characteristic curve; VCTE, vibration-controlled transient elastography; MRI, magnetic resonance imaging.
Authors’ contribution
KHC and WKC drafted the manuscript and edited it for important intellectual content. Both authors reviewed and agreed with the content of the final manuscript.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Hepatocellular carcinoma surveillance in non-alcoholic fatty liver disease – who and how?

Margaret LP Teng¹, Darren Jun Hao Tan², Cheng Han Ng², and Daniel Q. Huang¹²

¹Division of Gastroenterology and Hepatology, Department of Medicine, National University Health System; ²Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Keywords: Hepatocellular carcinoma; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Surveillance

Non-alcoholic fatty liver disease (NAFLD) is the fastest-growing cause of hepatocellular carcinoma (HCC) globally.¹ The burden of NAFLD-related HCC is predicted to increase further, in tandem with the obesity epidemic.² NAFLD encompasses a spectrum of histological severity, ranging from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH), which can consequently progress to liver fibrosis and cirrhosis.³ One of the strongest risk factors for NAFLD-HCC is fibrosis stage,⁴ and a prospective multi-centre study found that incidence of HCC per 100 person-years increased with fibrosis stage.⁵

Surveillance is associated with early detection of HCC and a higher likelihood of receiving curative treatment.⁶ Patients with early HCC are more likely to be eligible for curative treatment such as ablation, surgical resection, or liver transplantation, with 5-year survival rates of >70%.⁷,⁸ As such, surveillance is linked with improved overall survival.⁹ Despite the rise in the incidence of NAFLD-HCC,¹⁰ key questions remain regarding HCC surveillance in NAFLD patients—namely who to survey, and how to survey these patients.

In a recent issue of Clinical and Molecular Hepatology, El Daahan et al.¹¹ highlight that HCC surveillance should be limited to NAFLD patients with cirrhosis, and there is currently no consensus regarding HCC surveillance in NAFLD patients without cirrhosis. Currently, the American Gastroenterology Association (AGA) and the European Association for the Study of the Liver (EASL) suggest that HCC surveillance may be considered selected non-cirrhotic NAFLD patients.¹²,¹³ The AGA clinical practice update recommends the consideration of HCC surveillance in patients with advanced ≥F3 fibrosis, and proposes specific cut-offs on non-invasive tests (NITs) for consideration of surveillance—specifically, liver stiffness measurement of 16.1 kPa on vibration-controlled transient elastography (VCTE) and 5 kPa on magnetic resonance elastography (MRE) are cut-off values at which patients should consider HCC surveillance.¹⁴ EASL guidelines recommend that HCC surveillance may be considered in patients with advanced fibrosis diagnosed either on biopsy or elastography and acknowledge that surveillance in non-cirrhotic NAFLD
patients remains unclear.33
To improve HCC surveillance in NAFLD, further efforts are
needed to improve assessment of fibrosis stage, and identify
NAFLD patients with advanced fibrosis at elevated risk of
HCC. At present, AGA and EASL advocate a sequential ap-
proach using NITs (fibrosis-index 4 [FIB-4] followed by VCTE)
to identify NAFLD patients with advanced fibrosis.14 A pro-
spective study involving 5 tertiary European centres showed
that this strategy was able to predict risk of liver-related
events, which included both complications of cirrhosis and
HCC.15 Longitudinal assessment of NITs has also been pro-
posed as a method of monitoring changes in fibrosis over
time and could facilitate early detection of progression to ad-
vanced fibrosis or cirrhosis.26,27 Additionally, there are emerg-
ing data that NITs have potential for HCC risk stratification in
NAFLD patients. Several studies in Asia and Europe have
found that elevated FIB-4 was associated with a substantially
increased risk of HCC over a median follow-up of 7–10
years.18–20 More research is required to evaluate whether i)
other NITs such as VCTE and MRE and ii) longitudinal infor-
mation on NITs are correlated with HCC risk.

Next, HCC surveillance should be individualised in NAFLD
patients without cirrhosis. Restricting HCC surveillance in
NAFLD to patients with cirrhosis could miss a significant pro-
portion of NAFLD patients who develop HCC. Compared to
HCC of other etiologies, a higher percentage of NAFLD-HCC
patients were non-cirrhotic (38.5% vs. 14.6%).19 This may have
contributed to lower HCC surveillance rates among NAFLD-
HCC patients, as nearly 40% of NAFLD-HCC patients would
not have had an indication for routine surveillance based on
current guidelines. However, extending existing society rec-
ommendations to all non-cirrhotic NAFLD patients has major
implications. The incidence of HCC in patients with non-cir-
rhotic NAFLD is low at approximately 0.1–1.3 per 1,000 per-
son-years.2 HCC surveillance in this large and rising popula-
tion of NAFLD patients is neither feasible nor cost-effective.

There is a wide heterogeneity of HCC risk in non-cirrhotic
NAFLD patients—apart from degree of fibrosis, other factors
such as genetic polymorphisms, age, gender, obesity, and
type 2 diabetes have been associated with HCC risk.4 It would
be more accurate to assess HCC risk directly, rather than ex-
trapolating HCC risk from fibrosis stage. Several genomewide
association studies have identified single nucleotide polymorphisms (SNPs) such as patatin-like phospholipase
domain-containing protein 3 (PNPLA3) which could be linked
to increased risk of HCC.31 Polygenic risk scores including
these SNPs have been found to improve detection of HCC
particularly in individuals with dysmetabolism, and was able
to predict HCC independently of presence of severe fibrosis
in NAFLD patients.22 It has been suggested that risk factors
and estimates of fibrosis stage could also be combined into
risk calculators or risk prediction models to identify NAFLD
patients at higher risk of HCC who would benefit from sur-
veillance.4,13 A HCC risk calculator comprising 7 parameters
derived from clinical characteristics and serum lab tests has
previously been developed in patients with NAFLD cirrhosis
to estimate HCC risk,22 although this has not been externally
validated. Another novel risk prediction model comprising
age, platelet count, serum aspartate aminotransferase, and
liver stiffness based on VCTE has demonstrated utility in pre-
diction of HCC risk in NAFLD patients.24

El Dahan et al.13 comment that the current method most
often utilized for HCC surveillance, ultrasound (US) +/- al-
phafetoprotein (AFP), is inadequate for early detection of
HCC. US has a relatively poor sensitivity of <50% for early de-
tection of HCC.23 Furthermore, patients with NASH cirrhosis
were found to be more likely to have limited visualisation on
ultrasound.26,27 Surveillance failure could be attributed to the
presence of subcutaneous fat, focal fatty infiltration, and het-
erogeneity of liver parenchyma, which hinder the identifica-
tion of smaller lesions.28 The AGA clinical practice update ad-
vises that the adequacy of ultrasound for HCC surveillance
should be documented, and if inadequate, other imaging
modalities such as computed tomography (CT) scan or mag-
netic resonance imaging (MRI) should be considered.12 As
mentioned by El Dahan et al.,13 numerous alternative imaging
techniques such as abbreviated MRI protocols have been
proposed, but at present, data on their utility and cost-effec-

Abbreviations:
NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; AGA, American Gastroenterology Association; EASL, European Association for the Study of the Liver; NITs, non-invasive tests; VCTE, vibration-controlled transient elastography; MRE, magnetic resonance elastography; FIB-
4, fibrosis-index 4; SNPs, single nucleotide polymorphisms; US, ultrasound; AFP, alphafetoprotein; CT, computed tomography; MRI, magnetic resonance imaging; AASLD, American Association for the Study of Liver Disease


Margaret LP Teng, et al.
HCC surveillance in NAFLD


Margaret LP Teng, et al.
HCC surveillance in NAFLD
tiveness is lacking.

Both AGA and American Association for the Study of Liver Disease (AASLD) support US +/- AFP, whereas EASL supports US alone for HCC surveillance.\textsuperscript{12,13,29} A meta-analysis found that pooled sensitivity for detection of early HCC improved from 45% with US alone to 63% with addition of AFP to US.\textsuperscript{25} EASL also mentions that combining AFP and US leads to a modest 6–8% increase in detection of HCC.\textsuperscript{13} Given the inadequate visualisation on US in NAFLD patients, the addition of AFP to US should be considered to maximise the possibility of detection of HCC. El Dahan et al.\textsuperscript{11} discuss alternative biomarker-based surveillance tools such as GALAD and other novel biomarkers. Recent evidence suggests that liquid biopsy techniques such as methylation profiling of circulating tumour DNA have the potential to improve detection rates and transform the future of surveillance.\textsuperscript{30}

In conclusion, a multi-pronged strategy is required to optimise HCC surveillance in NAFLD patients. Improved risk stratification of NAFLD patients who might warrant HCC surveillance, as well as the adoption of more accurate biomarker- or imaging-based surveillance modalities may help address the challenges of HCC surveillance in NAFLD.

**Authors’ contribution**

Conceptualisation and Design: Margaret LP Teng, Daniel Q. Huang. Acquisition of Data, Analysis and Interpretation of Data: All authors Writing – original draft: Margaret LP Teng, Daniel Q. Huang. Writing – revision and final approval of version to be published: All authors.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

The effect of moderate alcohol consumption on nonalcoholic fatty liver disease

Ji-Won Park and Ki Tae Suk

1Institute for Liver and Digestive Diseases, Hallym University, Chuncheon; 2Department of Internal Medicine, Hallym University Sacred Heart Hospital of Hallym University Medical Center, Anyang; 3Department of Internal Medicine, Chuncheon Sacred Heart Hospital of Hallym University Medical Center, Chuncheon, Korea

Keywords: Alcohol; Nonalcoholic fatty liver disease; Moderate; Consumption

Nonalcoholic fatty liver disease (NAFLD) has emerged as a major cause of chronic liver disease worldwide since its first description in 1980 by Jurgen Ludwig and colleagues. The global prevalence has increased steadily over the last decade, reaching 25–30%.[2] NAFLD is interlinked with features of metabolic dysfunction including obesity, insulin resistance, type 2 diabetes mellitus, and dyslipidemia.[3] Diagnostic tests such as the fibrosis-4 index, as a first-line test, transient elastography, and blood testing can be used to differentiate high risk subjects in NAFLD patients.[4] The exclusion of significant alcohol consumption (more than 30 g/day in men and 20 g/day in women) is a prerequisite criterion for NAFLD diagnosis. Significant alcohol consumption typically follows the threshold suggested in guidelines of scientific association recommendations,[5,6] and the distinction between NAFLD and alcoholic liver disease relies on patient statements concerning alcohol consumption. This artificial classification is far from perfect because widespread issue of mild to moderate alcohol intake exists with metabolic derangements in patients diagnosed with “NAFLD”. The interaction between NAFLD and alcohol consumption has remained controversial over the last few years, in particular, the effects of moderate alcohol consumption on NAFLD is ill-defined.[7]

The definition of moderate alcohol consumption slightly differed from study to study, usually suggested as drinking in excess of the recommended limits for safe alcohol consumption. The safe levels of alcohol consumption suggested by the European Association for the Study of the Liver (EASL) and the American Association for the Study of the Liver (AASLD) are 30 g/day in men and 20 g/day in women, whereas, the Asian Pacific for the Study of the Liver (APASL) proposed a more cautious threshold of <20 g/day and 10 g/day in men and women, respectively.[8,9] However, with regards to the risk for advanced liver disease, prospective studies from the general population suggest that no safe limit of alcohol use exists.[10,11] In addition to alcohol quantity, alcoholic beverage type, drinking patterns, lifestyle patterns, and dietary constituents are important in NAFLD. These confounding factors provide complexity in the interpretation of previous studies.
There have been some studies suggesting a beneficial effect of moderate alcohol consumption with the occurrence and progression of NAFLD. However, most of these studies are cross-sectional studies and therefore cannot address the temporal relationship or causality between moderate alcohol consumption and NAFLD.

Looking at relatively well-characterized longitudinal cohort studies, Ekstedt et al. assessed weekly alcohol consumption at baseline and follow-up. In addition, heavy episodic drinking defined as >60 g/day in men and >48 g/day in women more than once a month was assessed. Although the study group was small (71 participants), they were biopsy-proven NAFLD patients and follow-up histology was investigated on an average of 13.8 years after the initial biopsy. They observed that heavy episodic drinking was independently associated with significant fibrosis progression, suggesting that low levels of alcohol consumption may cause fibrosis progression in NAFLD, especially when the drinking pattern exhibits intermittent ingestion of a significant amount of alcohol. With a similar aim, Ajimera et al. conducted a longitudinal cohort study using a NAFLD population taken from the nonalcoholic steatohepatitis clinical research network (CRN) trials. A total of 285 patients were included in the study, and the changes in NAFLD histology were evaluated using paired liver biopsies collected on an average of 3.9 years later. Modest alcohol consumption (defined as ≤2 drinks/day) was associated with less improvement in steatosis and aspartate transaminase levels and lower odds of nonalcoholic steatohepatitis resolution compared with consistent nondrinking individuals. In both the Ekstedt and Ajimera studies, ‘moderate drinkers’ included very low levels of alcohol consumption. Furthermore, a significant number of patients with NASH at baseline was present with this proportion being over 50% in both studies. Therefore, further studies are necessary to clarify the effect of “standardized” moderate alcohol consumption on a general NAFLD population. The study by Chang is valuable as the research meets these requirements. This prospective cohort study was performed in 58,927 young and middle-aged Korean adults with NAFLD and low fibrosis scores who were followed for a median of 4.9 years. Moderate drinkers were defined as 10–29.9 g/day and 10–19.9 g/day for men and women, respectively. The progression of NAFLD was assessed using noninvasive blood-based markers such as the NAFLD fibrosis score and Fibrosis-4 Index. They demonstrated that moderate alcohol consumption was independently associated with worsening fibrosis markers. There is another longitudinal study using the Finnish National Health Surveys (FINRISK, Health 2000) cohort. Åberg et al. determined that in subjects with fatty liver disease (defined as a fatty liver index ≥60), consuming 10–19 g/day of alcohol in general or 0–9 g/day as nonwine alcoholic beverages increased the risk for future advanced liver disease. Additionally, only among nonsmoking subjects, moderate alcohol consumption was associated with a reduced risk for cardiovascular disease events.

Based on the latest available longitudinal data, any amount of alcohol, even at low levels, cannot be encouraged in NAFLD patients. However, individual susceptibility to alcohol induced liver injury is substantially variable and the alcohol dose required to impact the disease course at individual patient levels may differ. Therefore, further investigations are required to enable individualized counseling regarding alcohol intake for each patient with NAFLD.

**Authors’ contribution**

Ji-Won Park contributed to write the manuscript. Ki Tae Suk contributed to study concept and revision.

**Acknowledgements**

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2020R1A6A1A03043026), Korea Institute for Advancement of Technology (P0020622), and Bio Industrial Technology Development Program (20018494) funded by the Ministry of Trade, Industry and Energy (MOTIE, Korea).

**Conflicts of Interest**

The authors have no conflicts to disclose.

---

**Abbreviations:**
NAFLD, nonalcoholic fatty liver disease; EASL, European Association for the Study of the Liver; AASLD, American Association for the Study of the Liver; APASL, Asian Pacific for the Study of the Liver; CRN, clinical research network
REFERENCES

1. Ayonrinde OT. Historical narrative from fatty liver in the nineteenth century to contemporary NAFLD - Reconciling the present with the past. JHEP Rep 2021;3:100261.
5. 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.
How to optimize the outcome of liver transplantation for non-alcoholic fatty liver disease

Byeong Geun Song and Dong Hyun Sinn

Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Keywords: Liver transplantation; Non-alcoholic fatty liver disease; Perioperative care

Along with global epidemics of obesity and type 2 diabetes mellitus (T2DM), the prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide. NAFLD is becoming one of the leading causes of liver transplantation (LT) both for end-stage liver disease (ESLD) and hepatocellular carcinoma. NAFLD is commonly associated with metabolic risk factors such as obesity and T2DM and poses unique challenges when considering LT. Therefore, comprehensive cardiovascular risk assessment and proper management of comorbid conditions are crucial in the LT evaluation process and post-LT management to improve outcomes. Liver transplantation can cure ESLD but not the underlying metabolic risk factors associated with NAFLD. Thus, long-term strategies to address these comorbidities are of importance in patient management. Furthermore, the use of postoperative steroids and other immunosuppressive agents frequently aggravates metabolic derangement and fosters development of obesity, insulin resistance, T2DM, hypertension, and dyslipidemia.

Because of the increasing prevalence of NAFLD and its impact on LT, efforts should be made to improve liver-related outcomes and prevent the development of metabolic-related complications following LT. However, present guidelines make no specific recommendation for LT recipients with NAFLD other than correction and optimal control of individual components of metabolic syndrome and cardiovascular risk factors. The European Association for the Study of the Liver (EASL) recommends that metabolic comorbidities be assessed and controlled in pre- and post-transplant settings. In the American Association for the Study of Liver Disease (AASLD) and American Society for Transplantation practice guidelines for LT, there are no NAFLD-specific directives other than that LT is an effective therapy for NAFLD cirrhosis. This comprehensive review article focuses on indications of LT for NAFLD, pre-LT risk assessment, management of patients on the waiting list, optimal management of metabolic disorder, immunosuppressive agent use, prevention of recurrent non-alcoholic steatohepatitis/NAFLD, and short- and long-term outcomes after LT.

Patients with NAFLD have a higher prevalence of cardiovascular disease with an increased risk of cardiovascular mortality than the general population because of common meta-
bolic risk factors and shared pathogenic pathways.\textsuperscript{9,10} Comprehensive cardiovascular risk assessment and testing are essential during the LT evaluation process, which warrants a multidisciplinary team approach, including cardiology, cardiac anesthesiology, and nutrition in addition to hepatology and transplant surgery, to appropriately stratify risk and optimize patient management.

Older age, higher Model for End-Stage Liver Disease score, and extreme body mass index, but not NAFLD itself, are risk factors for lower rate of survival after LT.\textsuperscript{11} However, NAFLD recurrence is frequent after LT because of persistence of metabolic risk factors and immunosuppressive agent use.\textsuperscript{2} Efforts should be made to achieve optimal control of metabolic comorbidities through a multidisciplinary team approach including nutrition, physical activity, and immunosuppressive agent modulation.

Unlike previous reviews, this study comprehensively describes research regarding association between post-LT outcomes and sarcopenia (pre-LT sarcopenia, post-LT sarcopenia, sarcopenic obesity, and changes in sarcopenic status). Sarcopenia has been increasingly reported to be associated with adverse outcome such as mortality, hospital stay, and infection after LT.\textsuperscript{12} Thus, nutritional status and physical activity assessment should be considered as part of the standard care. Also, specific recommendations and practical advice on diet and physical activity would be useful and relevant from a clinical point of view and have been briefly covered by another review article.\textsuperscript{13}

This review systematically describes management and therapeutic options to improve long-term outcomes with a particular emphasis on correction and control of metabolic comorbidities. Considering the growing impact of NAFLD on all aspects of LT, this review will provide useful information to optimize patient management in LT for NAFLD.

Authors’ contribution

Manuscript draft: Byeong Geun Song. Critical revision of manuscript: Dong Hyun Sinn.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES


The independent effect of exercise on biopsy-proven non-alcoholic fatty liver disease: A systematic review

Young-Joo Jin
Department of Internal Medicine, Inha University Hospital, Inha University School of Medicine, Incheon, Korea

Keywords: Exercise; Non-alcoholic fatty liver disease; Non-invasive test

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver diseases from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH), leading to fibrosis, cirrhosis, and even to hepatocellular carcinoma. The overall global prevalence of NAFLD is about 25% and steadily rising. However, to date, no drug has been approved for the treatment of NAFLD. Lifestyle modification, including exercise, weight reduction, and diet control is known to be the only accepted treatment for NAFLD. However, the independent effect of exercise on biopsy-proven NAFLD remains controversial.

This issue of the Clinical and Molecular Hepatology carried the first systemic review of published literature by Chen et al. for evidence on the independent effects of exercise on histological or non-invasive test (NIT) outcomes in patients with biopsy-proven NAFLD. The systemic review includes seven interventional and two observational studies. In this review, histologic endpoints were evaluated in six studies including two randomized controlled trials (RCTs), one non-RCT, one uncontrolled study, and two cross-sectional studies. Two RCTs failed to demonstrate the independent impact of exercise on histological improvement in the absence of weight reduction or diet intervention. On the other hand, the non-randomized interventional studies showed that exercise could reduce hepatocyte ballooning and liver fibrosis. However, these studies were limited by the absence of separate NASH-related data and by the uncontrolled study design. Moreover, Chen et al. did not analyze the difference between the effects of aerobic and anaerobic exercise on NAFLD. In the previous RCT involving subjects with clinically defined NAFLD, Hallsworth et al. showed that resistance exercise improves NAFLD regardless of changes in body weight. Therefore, the results would have been more meaningful if Chen et al. had also confirmed an independent effect according to the type of exercise on biopsy-proven NAFLD.

With regard to NIT, three RCTs and two non-RCTs assessed the independent effect of exercise on biopsy-proven NAFLD for hepatic steatosis, steatohepatitis, and liver fibrosis.
One RCT published by Rezende et al.\textsuperscript{17} used transient elastography as an NIT for the evaluation of the benefits of exercise in NAFLD patients with hepatic steatosis and fibrosis. Although this study is the only RCT to use transient elastography to assess the independent benefit of exercise on biopsy-proven NAFLD, aerobic exercise failed to demonstrate significant improvement of hepatic steatosis or fibrosis severity in this study.\textsuperscript{17} On the other hand, interestingly, other studies using magnetic resonance imaging-proton density fat fraction (MRI-PDFF) to measure outcomes have demonstrated improvement in hepatic steatosis due to exercise.\textsuperscript{18,19} However, there were no significant changes of the serum biomarkers for liver fibrosis and steatohepatitis.\textsuperscript{12,18,19} Given that MRI-PDFF is an accurate diagnostic method for hepatic fat over the entire liver and that it is a repeatable and reproducible quantitative examination method,\textsuperscript{20-23} this result is clinically significant.

To analyze the independent role of exercise, it is important to strictly control potential bias associated with the intensity, frequency, and type of exercise between eligible studies. However, it is not easy to completely control these variables. This would inevitably be a limitation of this study.\textsuperscript{9} It is also regrettable that a meta-analysis was not included in this systematic review.\textsuperscript{9} This is an area that needs to be supplemented through further research in the future.

Nonetheless, this study\textsuperscript{9} highlights the need for additional research to assess the independent role of exercise in the improvement of histologic and clinical biomarkers in patients with biopsy-proven NAFLD.

Acknowledgements
This was supported by Inha University Research Grant.

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES


Abbreviations:
NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NIT, non-invasive test; RCT, randomized controlled trials; MRI-PDFF, magnetic resonance imaging-proton density fat fraction


Single-cell phenotypes of peripheral blood immune cells in early and late stages of non-alcoholic fatty liver disease

Kathryn Jane Waller1,*, Hajar Saihi1,*, Wenhao Li1,*, James Hallimond Brindley1, Anja De Jong1, Wing-kin Syn2,3,4, Conrad Bessant5,6, and William Alazawi1

1Barts Liver Centre, Blizard Institute, Queen Mary University of London, London, UK; 2Division of Gastroenterology and Hepatology, Medical University of South Carolina, Charleston, SC, USA; 3Department of Physiology, Faculty of Medicine and Nursing, University of the Basque Country, Universidad del Pa S Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Leioa, Spain; 4Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, MO, USA; 5Centre for Computational Biology, Life Sciences Initiative, Queen Mary University of London, London; 6School of Biological and Chemical Sciences, Queen Mary University of London, London, UK

Graphical Abstract

Using CyTOF and bioinformatic methods to compare circulating immune cell changes in NAFLD

Study Highlights

- In this study, we studied the differences between blood immune cells in people with different stages of non-alcoholic fatty liver disease.
- We used cytometry by time of flight (CyTOF) with bioinformatics analysis to detect previously uncharacterizable changes in peripheral immune cells in early and late stages of non-alcoholic fatty liver disease.
- We found that blood immune cells, particularly T and B cells, NK cells, and monocytes change in NASH and become less active. The pathogenic role of immune cells in this condition warrants further attention.
INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. NAFLD affects approximately 25% of the Western population and represents a spectrum of liver disease, including steatosis, non-alcoholic steatohepatitis (NASH), which can lead to fibrosis, and in some patients, cirrhosis, liver failure, and liver cancer. Currently, NASH can only be distinguished from steatosis using liver biopsy, to grade and stage histological features of liver injury, inflammation, and fibrosis. Immune and inflammatory cell infiltration, together with hepatocyte injury, are the pathological hallmarks of non-alcoholic steatohepatitis (NASH). However, little is known about the composition and functional status of circulating immune and inflammatory cells in patients with NASH, particularly in the pre-cirrhotic stages. This is despite the fact that peripheral blood can be readily sampled and may give diagnostic and therapeutic in-

Background/Aims: Immune and inflammatory cells respond to multiple pathological hits in the development of non-alcoholic steatohepatitis (NASH) and fibrosis. Relatively little is known about how their type and function change through the non-alcoholic fatty liver disease (NAFLD) spectrum. Here we used multi-dimensional mass cytometry and a tailored bioinformatic approach to study circulating immune cells sampled from healthy individuals and people with NAFLD.

Methods: Cytometry by time of flight using 36 metal-conjugated antibodies was applied to peripheral blood mononuclear cells (PBMCs) from biopsy-proven NASH fibrosis (late disease), steatosis (early disease), and healthy patients. Supervised and unsupervised analyses were used, findings confirmed, and mechanisms assessed using independent healthy and disease PBMC samples.

Results: Of 36 PBMC clusters, 21 changed between controls and disease samples. Significant differences were observed between disease stages with changes in T cells and myeloid cells throughout disease and B cell changes in late stages. Semi-supervised gating and re-clustering showed that disease stages were associated with fewer monocytes with active signalling and more inactive NK cells; B and T cells bearing activation markers were reduced in late stages, while B cells bearing co-stimulatory molecules were increased. Functionally, disease states were associated with fewer activated mucosal-associated invariant T cells and reduced toll-like receptor-mediated cytokine production in late disease.

Conclusions: A range of innate and adaptive immune changes begin early in NAFLD, and disease stages are associated with a functionally less active phenotype compared to controls. Further study of the immune response in NAFLD spectrum may give insight into mechanisms of disease with potential clinical application. (Clin Mol Hepatol 2023;29:417-432)

Keywords: NAFLD; Mass cytometry
sights into disease—two areas in which clinical progress is limited by our understanding of the mechanisms that drive NASH.\textsuperscript{4,5} 

Current dogma is that a background of metabolic co-morbidity, genetic, dietary, and environmental factors predispose to multiple pathogenic hits that determine progression through the NAFLD spectrum. These hits include lipid-mediated hepatocyte injury through oxidative stress, effects of obesogenic diets on intestinal epithelial function and the microbiome which together activate damage-signalling pathways, and innate inflammatory responses.\textsuperscript{6} Lipid accumulation is believed to trigger oxidative stress in hepatocytes, generating damage associated molecular patterns that are sensed by the toll-like receptor (TLR) family. TLRs also detect gut-derived pathogen associated molecular patterns, such as lipoteichoic acid, lipopolysaccharide, and flagellin (ligands for TLRs 2, 4, and 5, respectively). TLR binding activates intracellular transcription factors including ERK, MAP kinases, and nuclear factor kappa B (NF-kB) and, indirectly, JAK-STAT proteins. Together, these release cytokines and chemokines drive inflammatory and fibrogenic processes and further compound metabolic dysfunction. 

To date, a limited number of studies have shown that peripheral blood pro-inflammatory Th1 cells are increased in patients with NASH,\textsuperscript{6,14} a phenotype also observed in people with obesity, who additionally have increased Th17 cells and reduced Th2 and regulatory T cells.\textsuperscript{7-9} Natural killer (NK) cells sampled from patients with NASH express higher levels of activation markers NKG2D, CD25, and CD69, and can produce higher levels of inflammatory cytokines in response to ex vivo stimulation compared to controls.\textsuperscript{10} Phenotypic shifts in monocytes have been described in people with NAFLD compared to controls, such as increase in intermediate and non-classical subsets and expression of cell surface TLRs, CD169, and CCR4.\textsuperscript{5,11-13} Fibrosis risk is increased in patients with hepatic steatosis and detectable autoantibodies or raised serum immunoglobulins,\textsuperscript{14} but the role of B cells that produce these antibodies has yet to be explored. Thus, emerging data indicate that lipid-related inflammation in the liver has the potential to activate immune responses, and we hypothesise that this leads to changes in immune cell phenotype and function. 

So far, studies of immune cell populations have largely depended on fluorescence-based techniques which, until recently, were limited by the number of cell types or functions that can be assessed in a single sample. Mass cytometry allows large numbers of markers to be assessed and analysed simultaneously at a single-cell level. Traditional gating-based approaches restrict analysis to known or pre-defined cell types, limiting the discovery of novel immune functions. Therefore, bioinformatic approaches are needed to combine prior knowledge with unsupervised high-dimensional clustering to interpret mass cytometry data where multiple features are examined together. 

We hypothesised that the peripheral composition and phenotype of peripheral blood immune cells differed between healthy controls, patients with steatosis, and those with NASH. We used single-cell cytometry by time of flight (CyTOF), together with a tailored unsupervised and semi-supervised bioinformatic pipeline, to report changes in total peripheral blood mononuclear cells (PBMCs) and at high resolution in T, B, and NK cells and monocyte-containing populations at early (steatosis) and late stages (NASH) of the NAFLD spectrum.

**MATERIALS AND METHODS**

**Patients and samples**

Patients and healthy volunteers were recruited from Barts Health NHS Trust and Queen Mary, University of London. Study protocols were approved by the East London and City Regional Ethics Committee (reference number 14/WA/1142) and performed in compliance with the Declaration of Helsinki. All participants gave their written informed consent. We included adult patients (age >18 years) with evidence of liver steatosis based on imaging (ultrasound, computed tomography, magnetic resonance imaging) or histology. Patients were excluded if they had any coexisting chronic liver disease diagnoses other than NAFLD, consumed alcohol greater than 14 units per week, or had clinical features of decompensated cirrhosis. Transient elastography was performed to measure liver stiffness according to standard clinical practice (reliable liver stiffness result based on successful reading rate >60% and interquartile range of all readings <30% of the median). Liver biopsy was performed in selected patients when clinically indicated. Each liver biopsy was reported by a single histopathologist (in routine clinical care) and were summarised according to the National Institutes of Health NASH...
PBMCs were sampled from 64 individuals (21 healthy, 43 with different stages of NAFLD), of which 19 samples (three healthy, 16 with NAFLD) were analysed by CyTOF. Ex vivo assays and conventional flow cytometry were used to confirm and extend CyTOF findings in the other 45 individuals (10 healthy, 35 with NAFLD). For CyTOF, those with NAFLD were subcategorised into steatosis (n=6) and NASH with fibrosis (n=10) according to liver histology or non-invasive fibrosis scores (Supplementary Table 1), giving three groups referred to as control, steatosis, and NASH, respectively.

**Cell separation**

PBMCs were separated by density gradient over Ficoll-Paque (GE Healthcare, Uppsala, Sweden).

**Mass cytometry**

We designed a panel of 36 metal-conjugated antibodies targeted against cell surface and intracellular proteins, selected to enable phenotyping and functional characterization (activation of intracellular signalling pathways, including the Toll-like receptor pathways, JAK-STAT signalling and NF-κB activation) of major immune cell subsets (https://www.biolegend.com/en-us/cell-markers) (Supplementary Table 2). Antibodies were either obtained pre-conjugated (Fluidigm, San Francisco, CA, USA) or conjugated in-house with trivalent metal isotopes using the MaxPAR antibody conjugation kit (Fluidigm). To select the most optimal metal isotope and antibody clone combination, the Maxpar Panel Designer tool was used (https://www.fluidigm.com/products-services/technologies/mass-cytometry) (Supplementary Table 2). One million PBMCs were washed with cell staining buffer (Fluidigm) before being incubated for 5 minutes at room temperature with Fc block (Biolegend, Munich, Germany). Surface marker antibodies were added for 30 minutes at room temperature. Cells were washed with cell staining buffer and fixed with 2% paraformaldehyde (PFA) (Sigma-Aldrich, St. Louis, MO, USA) for 10 minutes, followed by two further washes in cell staining buffer, and then permeabilised with 90% methanol for 30 minutes on ice. Cells were washed once with phosphate-buffered saline (PBS) and once with cell staining buffer, and then stained with intracellular antibodies for 45 minutes at room temperature. Cells were washed with FoxP3 permeabilisation buffer (eBioscience, San Diego, CA, USA) and stained with FoxP3 for 30 minutes at room temperature. Cell-ID Intercalator-Ir was diluted with Fix and Perm Buffer (Fluidigm) to 100 nM, added to each tube, and then left overnight or for a maximum of 3 days at 4°C. Cells were washed twice with ultrapure water (Milli-Q; Millipore Corporation, Bedford, MA, USA), added to an aqueous suspension of normalization beads (Fluidigm), and filtered through a 35-μm membrane prior to mass cytometry analysis.

**Flow cytometry**

Surface staining was performed on cells washed with FACS buffer (PBS, 2% FCS, 0.05% sodium azide, 0.5 mM EDTA). Dead cells were detected with Zombie NIR™ Fixable Viability Kit (Biolegend) according to the manufacturer’s protocol. Cells were then stained for 20 minutes at room temperature with antibody mixes including the following: CD3-PerCPCy5.5, CD3-PerPCCy5.5, CD4-FITC, CD4-PECy7, CD8-PerPCCy5.5, CD14-PerPCCy5.5, CD16-FITC, CD45RO-PE, CD45RA-APC, CCR6-PE, CXCR3-APC, and HLA-DR-PECy7 (all from Biolegend). For intracellular staining, cells were washed after surface staining, fixed with 2% PFA for 15 minutes at RT, and washed with permeabilization buffer (FoxP3 eBioscience buffer). Cells were then intracellular stained for 30 minutes at RT with antibody mixes that were made in permeabilization buffer, including interferon (IFN)γ-PB and IL-4-AF647 (all from Biolegend). Cells were washed with FACS buffer and acquired using a BD Canto II. All experiments were analysed using FlowJo v10.4 (Becton, Dickinson and Company, Ashland, OR, USA).

**CyTOF bioinformatic analysis**

All bioinformatics software package versions used in this pipeline are outlined in Supplementary Table 3.

**Pre-processing, batch correction, and dimension reduction**

Normalised (Fluidigm, normalisation passport EQ-P13H2302 version 2) live single-cell cisplatin-negative FCS files were exported from Cytobank™ and converted into CSV format using the ‘flowCore’ package. Median marker intensities of all live cisplatin negative cells were transformed using
the hyperbolic sine transform (arcsinh) with a cofactor of 1. We subsampled an equal number of 500,000 cells from each condition (healthy, steatosis, and NASH) and pooled these into a single data frame comprising 1.5 million cells by 36 marker dimensions. Cells were then batch corrected for variation using the CyCombine method; in summary, we used an 8x8 grid and apply the 'scale' normalisation approach. Dimension reduction was carried out the batch the corrected transformed cells using the Uniform Manifold Approximation and Projection (UMAP) algorithm. UMAP parameters were set to 15 nearest neighbours and a minimum distance of 0.1.

Immunophenotypic cell subset identification

Phenotyping by accelerated refined community-partitioning (PARC) was applied to carry out unsupervised identification of immunophenotypic cell subsets on all batch-corrected cells. Default PARC parameters were used. Re-clustering of manually gated populations were carried out for semi-supervised high-resolution analysis; in these instances, the 'small_pop' parameter of PARC was set to 5,000 cells. Cluster abundances across patients were analysed via principal component analysis. Input to the principal component analysis was the abundance of each cluster for each patient. Cluster abundances were z-score normalised and visualised using the 'heatmap.2' package in R to visualise the abundance of each immunophenotypic cluster for each patient.

Statistical analysis

The diffcyt pipeline was used to carry out differential immunophenotypic cluster abundance analysis (diffcyt-DAedgeR) in R using the previously identified PARC clusters. A design matrix was created to conduct three comparisons: NASH relative to control, NAFLD relative to NASH, and NASH relative to NAFLD. Cluster counts were normalised using weighted trimmed mean of M-values to account for composition effects of cluster counts across patient groups. Each contrast resulted in an output table (produced using the top Table function) with summary statistics on the log2 fold change of the normalised cluster abundances and Benjamini-Hochberg adjusted P-values to account for multiple testing. A controlled false discovery rate (FDR) level of <0.05 was considered statistically significant.

Data visualisation

Bar plots and cluster-marker bubble diagrams were generated using the ‘ggplot2’ package in R. Hierarchical clustering was carried out using the ‘ggdendro’ package in R.

RESULTS

NAFLD disease stage characterised by changes in peripheral immune cell phenotype

Compared to the steatosis group, the median age of the NASH group was older (61 vs. 50 years, P=0.03) with higher prevalence of type 2 diabetes (70% vs. 17%, P<0.0001). The median body mass indices in steatosis and NASH groups were significantly higher compared to the control group (31.0 kg/m^2 and 28.9 kg/m^2, respectively, compared to 23.2 kg/m^2, P=0.004). Immune cell cluster distribution differed between the control, steatosis, and NASH groups (Fig. 1A, B). Principal component analysis showed separation of the control individuals away from NASH (Fig. 1C), with steatosis samples lying in an intermediate position.

To study known cell types in different stages of disease, we applied a pre-defined classical gating strategy (Supplementary Fig. 1) to the mass cytometry-acquired single cell dataset. We found significant differences in the proportions of live cells of Th1, Th2, cytotoxic T cells, and myeloid-derived suppressor cells (Supplementary Fig. 2). We also confirmed the changes in Th1 and Th2 cells in an independent group of samples from fifteen patients with NASH and fibrosis and eight healthy controls using flow cytometry (Supplementary Fig. 3, Supplementary Table 1).

To gain detailed insight into the changes in immune cells in different stages of disease, we applied unsupervised methods to cluster cells according to relative expression (Fig. 2A circle size) and median intensity (Fig. 2A circle colour) of each antibody signal. Twenty-three out of 36 clusters changed significantly in abundance between disease states. Four clusters (0, 13, 20, and 30) increased in steatosis and NASH groups compared to the control group (Fig. 2B). A further four clusters (9, 25, 26, and 34) increased in NASH group compared to steatosis group. Conversely, six clusters (4, 16, 19, 21, 23, and 27) had fewer cells in NASH group compared to steatosis.
group. Some changes were only statistically significant when comparing NASH group with the control group (17, 24, and 28 increased; 1, 11, 14, 15, 18, and 29 decreased).

**Number and function of known cell types change in NAFLD**

Based on the pattern of marker expression, 14 clusters could be assigned to known existing cell types: seven bore T cell markers, four myeloid, two NK cell, and one mucosal-associated invariant T (MAIT) cell markers. Among the clusters that expressed myeloid markers, cluster 13 (increased in steatosis) expressed HLA-DR, TLR2, and TLR5 strongly, but had lower levels of CD14, phosphorylated (p)NF-κB, pCREB, and arginase. Cluster 19 (reduced in NASH) also expressed HLA-DR strongly and had higher levels of pNF-κB, pCREB, arginase, pSTAT2, and CD14, with low levels of CD16. Cluster 14 cells expressed CD3, CD161, and TCRVa7.2 with high levels of

---

**Figure 1.** (A) PARC live cell clusters visualised on a two-dimensional UMAP plot. UMAP plots show changes in PARC clusters at the single cell level in the control, steatosis, and NASH patients. In the combined UMAP, grey represents single cells in steatosis and NASH that overlap with control. Yellow represents unique cells in steatosis compared to control, and red represents unique cells in NASH compared to control. An equal number (500,000) of cells from each group (control, steatosis, NASH) have been subsampled. (B) Heatmap representing z-score normalised cluster abundance across all patient groups and all live cell clusters. Rows represent the z-normalised cluster abundance across all clusters for each patient, columns patient expression profiles across clusters. Columns are coloured by either green (control patients), blue (steatosis patients), and pink (NASH patients). (C) PCA plot based on cluster abundance across patient groups. Green circles represent control patients (n=3), blue circles represent steatosis patients (n=6), and red circles represent NASH patients (n=10). PARC, phenotyping by accelerated refined community-partitioning; UMAP, Uniform Manifold Approximation and Projection; NASH, non-alcoholic steatohepatitis; PCA, principal components analysis.
pNF-xB and pCREB, indicating that the cluster contained transcriptionally active MAIT cells and was significantly less abundant in NASH group compared to steatosis and control groups.

Of the seven T cell groups, two clusters (4 and 17) that expressed high-intensity CD8 (marker of cytotoxic T cells) and three clusters (0, 9, and 34) that expressed high-intensity CD3, CD4, and CXCR3 (Th1 markers) (Fig. 2B) increased in disease states, which was consistent with the findings of the manual gating of mass cytometry and flow cytometry (Supplementary Figs. 2, 3). We identified Cxc3/Gsc6 Th1 cells in a search of the Liver Cell Atlas (https://www.livercellatlas.org/datasets_NAFLDmouse.php), derived from single-cell analysis of Western diet-fed mouse liver tissue (Supplementary Fig. 4). We hypothesised that lipid-mediated hepatocyte injury could drive a 'skew' in T helper cell differentiation towards a Th1 phenotype. To test this, we incubated hepatocyte-like HepG2 cells with a 2:1 combination of palmitic acid and oleic acid (1 mM and 0.5 mM) for 24 hours to model lipid-induced hepatocyte injury. Naïve T cells isolated from healthy controls were then incubated in media supplemented with supernatant from the fatty-acid treated HepG2 cells. This resulted in a higher proportion of CD3+CD4+CXCR3+ cells (Supplementary Fig. 5A) and higher expression levels of the Th1 cytokine IFNγ (Supplementary Fig. 5B) compared to the naïve T cells cultured in supernatant from control-treated HepG2 cells. Taken together, these data indicate that lipid-mediated hepatotoxicity generates a milieu that can induce naïve T cells to differentiate towards Th1 cells as identified by CyTOF.

Collectively, our data demonstrate that shifts in broad immune cell types occur in both steatosis and NASH. However, this combination of traditional analysis and unsupervised clustering does not make use of the granularity of the large number of markers included in the panel, leaving nine clus-

Figure 2. (A) Balloon plot representing relative and median intensity profiles across live cell clusters for all markers. The size of each circle represents the relative expression of each marker across all clusters. Each circle is coloured based on the median intensity of each marker in a given cluster. Markers with shared expression profiles across clusters are located together (right dendrogram) and clustering of cells with shared median marker expression profiles across markers are located together (top dendrogram). Dark brown circles represent high relative expression and high median intensities. (B) Differential cluster abundance analysis between control, steatosis, and NASH patients. A bar plot to show the log fold change in cluster abundance. Log fold change refers to the change in cluster abundance between steatosis patients compared to control patients, NASH patients compared to control patients, and NASH patients compared to steatosis patients, respectively. Coloured bars represent clusters that reach statistical significance (FDR <0.05). Grey bars represent clusters that do not reach statistical significance (FDR >0.05). NASH, non-alcoholic steatohepatitis; FDR, false discovery rate.
Changes in CD3⁺ T cell clusters throughout the NAFLD spectrum

The abundance of eight out of 18 clusters of CD3⁺ cells (Fig. 3A, B) changed between the control, steatosis, and NASH groups (Fig. 3B, D). The greatest number of cluster differences was observed between NASH group and controls; although many of these changes were apparent in steatosis group versus controls, they did not reach statistical significance. CD3⁺ cluster 13 (Fig. 3C) expressed the highest levels of CD161 and TCRVa7.2 (MAIT cell markers); and, consistent with the unsupervised analysis, was lower in patients with NASH compared to the controls. We confirmed this reduction in activated MAIT cells in the blood sampled from an independent group of 14 patients with NASH and fibrosis or steatosis and seven healthy controls using classical fluorescence flow cytometry (Supplementary Fig. 6).

Expressions of CD4 and CD8 were largely reciprocal across clusters (Fig. 3C). Disease state-related changes were observed in five CD4-expressing (0, 3, 4, 14, and 17) and three CD8-expressing (2, 13, and 16) clusters. CD4-expressing cluster 17, characterised by high levels of CD4 and T-bet, increased in both steatosis and NASH comparisons. Since this cluster included CXCR3- and CCR6-expressing cells, it may contain different groups of T helper cell, although these cells may be less active with lower levels pNF-kB, pERK1&2, and pSTATs. In keeping with this increase in cells with low signalling activity, CD4-expressing clusters (4 and 14) that co-expressed pERK1&2, pSTAT1, and decreased in abundance. Taken together, both the activation status of T cells as well as their phenotype should be taken into account when distinguishing between NASH, steatosis, and healthy patients.

Changes in CD3⁺CD19⁺ B cells in patients with steatosis and NASH compared to controls

Compared to controls, four clusters of CD3 CD19⁺ cells (Fig. 4A, B) changed in abundance in NASH group, but not in steatosis (Fig. 4B, D). All CD3 CD19⁺ clusters also expressed high intensity of HLA-DR indicative of B cells (Fig. 4C). Clusters 0 and 8 that increased were characterised by higher levels of the following: co-stimulatory molecule CD86; memory markers CD11b, CXCR3, CD11c, CD33; and TLRs 2, 5, and to a lesser extent, TLR4. Clusters that decreased in abundance expressed higher levels of pCREB and pERK1&2, which are related to the activation status. In the mouse Liver Cell Atlas, CD33 was principally expressed in neutrophils, plasmacytoid dendritic cells, and monocyte derived cells, but not in B cells (Supplementary Fig. 7).

Changes in CD3⁺CD19⁺CD14⁺CD56⁺ NK cells in steatosis and NASH

The abundance of five out of 11 clusters of CD3 CD19⁺CD14⁺CD56⁺ cells changed between control, steatosis, and NASH groups (Fig. 5). Clusters 0 and 8 were increased in steatosis group compared to the control group, and cluster 6 increased in NASH group compared to steatosis group—all three characterised by low expression of the activation marker NKP30. Reduced numbers of cells were observed in clusters 5 (steatosis) and 9 (NASH); both characterised by higher expression of phosphorylated signalling mediators: pERK, pCREB, pP38, and pSTAT1, 2, 3, and 5. This suggests that, as with T cells, more advanced stages of NAFLD are associated with higher numbers of less active NK cells.

Changes in CD3⁺CD19⁺CD14⁺ cell clusters in patients with steatosis compared to controls

We identified nine clusters of CD3 CD19⁺CD14⁺ cells (Fig. 6A, C), four of which (expressing high levels of HLA-DR) changed in abundance between control, steatosis, and NASH groups (Fig. 6B, D). We also observed variation in the expression lev-
els of CD14 and CD16 between the clusters that changed significantly, which went beyond the definitions of classical, intermediate, and non-classical subtypes. Cluster 6 was more abundant in NASH group compared to controls, and was characterised by less phosphorylated pSTAT2, MAPKAP2, pP38, pNF-κB, and pCREB staining. Cluster 4 (reduced in steatosis) and clusters 2 and 5 (reduced in NASH) were characterised by high expression and intensity of signalling mediators and TLRs (TLR2, TLR4, and TLR5). These findings are in keeping with, and give greater detail to, the observations in

Figure 3. (A) CD3⁺CD19⁻ gating strategy. (B) CD3⁺CD19⁻ clusters visualised on a two-dimensional UMAP plot. UMAP plots show changes in CD3⁺CD19⁻ single cell clusters between control, steatosis, and NASH patients. Each colour represents a single cluster. Circle labels indicate cluster numbers. (C) Balloon plot representing relative and median intensity profiles across CD3⁺CD19⁻ clusters for all markers. The size of each circle represents the relative expression of each marker across all clusters. Each circle is coloured based on the median intensity of each marker in a given cluster. Markers with shared expression profiles across clusters are located together (right dendrogram), and clustering of cells with shared median marker expression profiles across markers are located together (top dendrogram). (D) Differential CD3⁺CD19⁻ cluster abundance analysis between control, steatosis, and NASH patients. A bar plot to show the log fold change in cluster abundance. Log, fold change refers to the change in cluster abundance between steatosis patients compared to control patients, NASH patients compared to control patients, and NASH patients compared to steatosis patients, respectively. Coloured bars represent clusters that reach statistical significance (FDR <0.05). Grey bars represent clusters that do not reach statistical significance (FDR >0.05). UMAP, Uniform Manifold Approximation and Projection; NASH, non-alcoholic steatohepatitis; FDR, false discovery rate.
the unsupervised analysis, and suggest that NASH is associated with circulating monocytes that are less active and perhaps less responsive to TLR-mediated stimulation. To test this hypothesis, we stimulated peripheral blood mononuclear cells isolated from healthy individuals or n=9 validation group patients with NASH and fibrosis with lipopolysaccharide (LPS) or flagellin. LPS- and flagellin-induced interleukin (IL)-6 and tumor necrosis factor (TNF)α production were sig-

Figure 4. (A) CD3\(^{+}\)CD19\(^{-}\) gating strategy. (B) CD3 CD19\(^{-}\) clusters visualised on a two-dimensional UMAP plot. UMAP plots show changes in CD3 CD19\(^{-}\) single cell clusters between control, steatosis, and NASH patients. Each colour represents a single cluster. Circle labels indicate cluster numbers. (C) Balloon plot representing relative and median intensity profiles across CD3 CD19\(^{-}\) clusters for all markers. The size of each circle represents the relative expression of each marker across all clusters. Each circle is coloured based on the median intensity of each marker in a given cluster. Markers with shared expression profiles across clusters are located together (right dendrogram), and clustering of cells with shared median marker expression profiles across markers are located together (top dendrogram). (D) Differential CD3 CD19\(^{-}\) cluster abundance analysis between control, steatosis, and NASH patients. A bar plot to show the log, fold change in cluster abundance. Log, fold change refers to the change in cluster abundance between steatosis patients compared to control patients, NASH patients compared to control patients, and NASH patients compared to steatosis patients, respectively. Coloured bars represent clusters that reach statistical significance (FDR <0.05). Grey bars represent clusters that do not reach statistical significance (FDR >0.05). UMAP, Uniform Manifold Approximation and Projection; NASH, non-alcoholic steatohepatitis; FDR, false discovery rate.
nificantly impaired in NASH and fibrosis patients compared to controls, as was NF-κB phosphorylation (Fig. 6E-H).

**DISCUSSION**

We used single-cell mass-cytometry together with a tailored unsupervised and supervised bioinformatics pipeline to detect a significant variation in innate and adaptive immune cell populations in the peripheral blood of patients with NAFLD. Patients and controls clustered separately, as well as semi-supervised high-resolution clustering showed phenotypic and functional heterogeneity within the known cell types. The main differences between steatosis and con-

---

**Figure 5.** (A) CD3 CD19 CD14 CD56+ gating strategy. (B) CD3 CD19 CD14 CD56+ clusters visualised on a two-dimensional UMAP plot. UMAP plots show changes in CD3 CD19 CD14 CD56+ single cell clusters between control, steatosis, and NASH patients. Each colour represents a single cluster. Circle labels indicate cluster numbers. (C) Balloon plot representing relative and median intensity profiles across CD3 CD19 CD14 CD56+ clusters for all markers. The size of each circle represents the relative expression of each marker across all clusters. Each circle is coloured based on the median intensity of each marker in a given cluster. Markers with shared expression profiles across clusters are located together (right dendrogram), and clustering of cells with shared median marker expression profiles across markers are located together (top dendrogram). (D) Differential CD3 CD19 CD14 CD56+ cluster abundance analysis between control, steatosis, and NASH patients. A bar plot to show the log, fold change in cluster abundance. Log, fold change refers to the change in cluster abundance between steatosis patients compared to control patients, NASH patients compared to control patients, and NASH patients compared to steatosis patients, respectively. Coloured bars represent clusters that reach statistical significance (FDR <0.05). Grey bars represent clusters that do not reach statistical significance (FDR >0.05).
Figure 6. (A) CD3 CD19 CD14⁺ gating strategy. (B) CD3 CD19 CD14⁺ clusters visualised on a two-dimensional UMAP plot. UMAP plots show changes in single cell CD3 CD19 CD14⁺ clusters between control, steatosis, and NASH patients. Each colour represents a single cluster. Circle labels indicate cluster numbers. (C) Balloon plot representing relative and median intensity profiles across CD3 CD19 CD14⁺ clusters for all markers. The size of each circle represents the relative expression of each marker across all clusters. Each circle is coloured based on the median intensity of each marker in a given cluster. Markers with shared expression profiles across clusters are located together (right dendrogram), and clustering of cells with shared median marker expression profiles across markers is located together (top dendrogram). (D) Differential CD3 CD19 CD14⁺ cluster abundance analysis between control, steatosis, and NASH patients. A bar plot to show the log, fold change in cluster abundance. Log, fold change refers to the change in cluster abundance between steatosis patients compared to control patients, NASH patients compared to control patients, and NASH patients compared to steatosis patients, respectively. Coloured bars represent clusters that reach statistical significance (FDR <0.05). Grey bars represent clusters that do not reach statistical significance (FDR >0.05). (E) Flow cytometry analysis of phosphorylated NFκB. PBMCs sampled from patients with NASH with fibrosis and healthy controls were stimulated with medium, LPS (1 µg/mL) or flagellin (1 µg/mL), for 15 minutes and analysed by flow cytometry. (F) IL-6 production, (G) TNFα production, and (H) IL-10 production measured by ELISA. PBMCs sampled from patients with NASH with fibrosis and healthy controls were stimulated with medium, LPS (20 ng/mL) or flagellin (100 ng/mL), for 24 h before supernatants were collected for ELISA.
trolo groups were increases in NK and T cells with low levels of phosphorylated transcription factors. The majority of differences were observed between NASH and steatosis; an increase in B cells expressing memory markers and co-stimulatory molecules and further increase in NK and T cells with low levels of phosphorylated transcription factors paralleled by increase in NK, T, MAIT and B cells and monocytes with high levels of phosphorylated transcription factors. Functionally, monocytes taken from patients with more advanced stages of disease were less responsive to TLR agonists LPS and flagellin, in keeping with the single-cell data. Lipid-mediated hepatotoxicity in vitro generated a milieu that induced healthy naive T cells to differentiate towards a Th1 phenotype, in keeping with our CyTOF and flow cytometry data in patients with steatosis and NASH and with murine data from the Liver Cell Atlas. Further mechanistic study is needed to confirm the drivers of Th1 differentiation in vivo.

Increasing evidence points towards a role for adaptive immunity in NASH pathogenesis. The inflammatory infiltrate in NASH contains B and T lymphocyte aggregates, and the size and number of these aggregates are correlated with the degree of inflammation and fibrosis. Patients with obesity with or without type 2 diabetes, who are at the highest risk of NAFLD, have increased numbers of circulating Th1 and Th17 cells and higher levels of Th1-promoting IFNγ with a reciprocal reduction in Th2 and regulatory T cells. Although few have studied peripheral immune cells in people with simple steatosis, numbers of naive IFNγ-expressing CD4+ T cells were increased in the peripheral blood of 20 patients with NASH, and high levels of IFNγ mRNA expression in CD4+ T cells were observed in 51 patients with biopsy-proven NASH. Consistent with this, we found that Th1 cells and CD8-expressing cytotoxic T cells were increased in patients with steatosis, and these remained elevated in NASH and fibrosis patients, albeit with lower levels of key phosphorylated signalling mediators, which may reflect an exhausted phenotype in NASH. This was consistent with recent findings of a novel subset of CXCR6+ CD8+ T cells with high expressions of both activation and exhaustion markers in NASH patients, which induced hepatocyte killing in an MHC-class-1-independent fashion via IL-15 and acetate in the liver. Haas et al. reported increase in activated cytotoxic CD8+ T cells in NASH, which accumulate in close proximity to steatotic and injured hepatocytes in the liver. They also reported that circulating and liver CD8+ T cells correlated strongly with histological hallmarks of NASH: ballooning and lobular inflammation. Together, our study adds to the literature which shows that CD8+ T cells play a role in mediating hepatic inflammation and hepatocyte cell death in NASH, independently of antigen recognition.

Despite this evidence of antigen-independent T cell activation, specific antigens that could elicit an adaptive immune response have been identified in obesity and in NASH. Oxidised phospholipids and aldehydes form antigenic adducts called oxidised stress epitopes, antibodies against which can be detected in approximately 40% of patients with NAFLD or NASH. The cellular infiltrate in NASH includes B lymphocytes, and selected depletion of B2 cells in mice results in mild NASH and less fibrosis. B cell-derived inflammatory mediators activate hepatic stellate cells and the reciprocal production of retinoic acid can promote B cell maturation into plasma cells. In NASH, we found increased B cells expressing CD33 along with co-stimulatory molecules, suggesting a role for antibody-producing B cells. However, it remains to be determined whether these B cells are clonal and whether a specific antibody-mediated response drives NASH. CD33 is a transmembrane receptor that is typically highly expressed on myeloid-committed cells, implicated in regulating cellular expansion, activation, and pro-inflammatory cytokine secretion. Increasing circulating CD33+ B cells have been reported in other systemic inflammatory conditions, including Behcet’s disease and sepsis, although CD33+ B cells were not identified in the murine Liver Cell Atlas. Further study is required to determine the pathogenesis of liver infiltrating B cell subsets in NASH.

We identified progressive changes in circulating MAIT cells; non-conventional innate-like T cells that express invariant T cell receptor (TCR) α-chain, composed of Va7.2-Ja33 and are restricted by the major histocompatibility complex class I-related molecule MR1. The current literature reports both protective and pathogenic roles in NASH. We observed reduced circulating MAIT cells frequency in NASH group compared to steatosis and control groups, extending previous observations in patients with high NAFLD activity scores. Similarly, reduced numbers of circulating MAIT cells accompanied by increased intra-adipose MAIT cell numbers have been reported in diabetes and obesity.

A recent study reported no significant change in the numbers of NK cells in patients with NAFLD, although there was an increase in NK cell activation marker NKG2D in patients.
with NASH and fibrosis. Other studies have found reduced CD56dim NK cell subset with increased exhaustion markers, including programmed death 1 and immunoglobulin-like transcript 2, in NAFLD patients compared to the controls. In the current study, although we did not have direct markers of exhaustion in our CyTOF panel, we found significant heterogeneity among NK cells with a reduction in the number of intracellular signalling mediators, including pERK, pCREB, pP38, and pSTATs 1, 2, 3, and 5, reflecting a less activated phenotype in NASH. Three clusters changed in abundance in steatosis, persisting through to NASH, and a further two clusters changed in NASH compared to steatosis. Given the emerging role of NK cells in modulating metabolic function and the known associations of NK cells with liver disease in viral hepatitis, more detailed study of this heterogeneous cell type is warranted.

Large numbers of myeloid cells reside in and patrol the liver, including Kupffer cells and blood-derived macrophages and dendritic cells. Recent single cell techniques have shown 14 subtypes of intrahepatic myeloid cells in advanced liver disease, including NASH. We found nine clusters of circulating CD14+ cell, of which four changed in abundance in disease. These changes went beyond the traditional classification of monocytes into three groups based on CD14 and CD16 expression and intensity. Therefore, to delineate monocyte differentiation in NASH, further subclassification with other cell markers may be required. Clusters with markers of activation were less abundant in patients with NASH and ex vivo, PBMCs sampled from patients with NASH produced lower amounts of TLR-mediated TNFα, IL-6, and IL-10 compared to the controls. This was similar to the findings in decompensated NASH cirrhosis, suggesting that changes in monocyte immunocompetence may begin earlier than those previously thought in NASH.

Our unsupervised and semi-supervised clustering methods address some of the limitations of traditional bi-axial gating approaches that require prior knowledge of immune cell populations. A significant advantage of using PARC as a clustering method was that we did not need to down-sample cells that could lead to information loss. The changes we observed by mass-cytometry were confirmed in independent samples analysed by fluorescence-based techniques, and the findings reported in earlier studies have been extended here. However, our study had a number of limitations. The cross-sectional nature of the study precludes causal conclusions to be drawn from our findings. People with NASH in this study were older and more obese than the individuals in the control group. Finally, a larger sample size would enable us to determine whether the changes we detect are related to the development of NASH or to metabolic inflammation observed in all patients with, for example, type 2 diabetes or obesity or early NASH, prior to the onset of liver fibrosis. We do not know the origin or mechanistic role of the cells detected in the pathogenesis of NASH. Our study focused on the peripheral immune compartment, and larger studies are needed to incorporate immune cells from the liver and adipose tissue combined with the approach and analytics used here to determine clonal and phenotypic relationships across tissue compartments. Whether the number and composition of immune cells can be used as non-invasive markers of disease stage or of response to treatment remains to be seen. However, better understanding of the function of the cells we have identified may lead to the discovery of soluble mediators that are more easily detected, which can be used for such clinical applications.

In conclusion, our study has demonstrated that innate and adaptive immune changes occur early in NAFLD and can be detected in the peripheral blood. We have used a tailored analytical pipeline and found heterogeneity among these cell types with distinct functional profiles. The role of the immune response in NAFLD warrants further attention.

Authors’ contribution
K.J.W., H.S., W.L. contributed equally to the manuscript. Data acquisition and analysis: K.J.W., H.S., W.L., J.H.B. Initial drafting of manuscript: K.J.W., W.L. Critical review and adaptation of the manuscript: A.D., C.B., W.K.S. and W.A. Study conception and supervision: W.A. and C.B. All authors approved the final version. Data, analytic methods, and study materials can be requested through corresponding author.

Conflicts of Interest
WA has received honoraria for speaking and consultancy from Gilead Sciences, Glaxosmithkline, Intercept and Coherus, and competitive funding from Gilead Sciences and Glaxosmithkline. He is supported by grant funding from the Medical Research Council. Other authors have no conflict of interest to declare.
SUPPLEMENTARY MATERIAL

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

REFERENCES


5. Lambrecht J, Tacke F. Controversies and opportunities in the use of inflammatory markers for diagnosis or risk prediction in fatty liver disease. Front Immunol 2021;11:634409.


8. Rau M, Schilling AK, Meertens J, Hering J, Weiss J, Jurowich C, et al. Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. J Immunol 2016;196:97-105.


Sungchul Choi1, Beom Kyung Kim2,3,4,*, Dong Keon Yon5,*, Seung Won Lee6,*, Han Gyeol Lee1, Ho Hyeok Chang1, Seoyeon Park1, Ai Koyanagi7,8, Louis Jacob7,9, Elena Dragioti10, Joaquim Radua11,12,13, Jae Il Shin14, Seung Up Kim2,3,4, and Lee Smith15

1Yonsei University College of Medicine, Seoul; 2Department of Internal Medicine, Yonsei University College of Medicine, Seoul; 3Institute of Gastroenterology, Yonsei University College of Medicine, Seoul; 4Yonsei Liver Center, Severance Hospital, Yonsei University Health System, Seoul; 5Department of Pediatrics, Kyung Hee University Medical Center, Kyung Hee University College of Medicine, Seoul; 6Department of Precision Medicine, Sungkyunkwan University School of Medicine, Suwon, Korea; 7Research and Development Unit, Parc Sanitari Sant Joan de Déu, CIBERSAM, Dr. Antoni Pujadas, Barcelona; 8Catalan Institute for Research and Advanced Studies, Pg. Lluís Companys 23, Barcelona, Spain; 9Department of Physical Medicine and Rehabilitation, Lariboisière-Fernand Widal Hospital, AP-HP, University Paris Cité, Paris, France; 10Pain and Rehabilitation Centre, and Department of Health, Medicine and Caring Sciences, Linkoping University, Linkoping, Sweden; 11Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), CIBERSAM, Instituto de Salud Carlos III, University of Barcelona, Barcelona, Spain; 12Centre for Psychiatric Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; 13Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK; 14Department of Pediatrics, Yonsei University College of Medicine, Seoul, Korea; 15Centre for Health, Performance, and Wellbeing, Anglia Ruskin University, Cambridge, UK

Graphical Abstract

Global burden of primary liver cancer and its association with underlying aetiologies, sociodemographic status, and sex differences from 1990-2019

Underlying aetiologies
1. Hepatitis B virus
2. Hepatitis C virus
3. Alcohol consumption
4. NASH/NAFLD
5. Other causes
Study Highlights

- The crude numbers of DALYs, deaths, and incident cases of liver cancer significantly increased during the study period; however, the Age-standardized DALY and mortality rates as well as incidence decreased.
- HBV was the leading cause of liver cancer DALYs, mortality, and incidence, followed by HCV, alcohol consumption, and NASH/NAFLD.
- In 2019, the high-income Asia-Pacific population recorded the highest Age-standardized DALY and mortality rates, followed by those of East Asia and Central Asia. High-income North American and Australasian populations also showed significant increases.

Background/Aims: Global distribution of dominant liver cancer aetiologies has significantly changed over the past decades. This study analyzed the updated temporal trends of liver cancer aetiologies and sociodemographic status in 204 countries and territories from 1990 to 2019.

Methods: The Global Burden of Disease 2019 report was used for statistical analysis. In addition, we performed stratification analysis to five quintiles using sociodemographic index and 21 geographic regions.

Results: The crude numbers of liver cancer disease-adjusted life years (DALYs) and deaths significantly increased during the study period (DALYs; 11,278,630 in 1990 and 12,528,422 in 2019, deaths; 365,215 in 1990 and 484,577 in 2019). However, the Age-standardized DALY and mortality rates decreased. Hepatitis B virus (HBV) remains the leading cause of liver cancer DALYs and mortality, followed by hepatitis C virus (HCV), alcohol consumption, and non-alcoholic steatohepatitis/non-alcoholic fatty liver disease (NASH/NAFLD). Although Age-standardized DALY and mortality rates of liver cancer due to HBV and HCV have decreased, the rates due to alcohol consumption and NASH/NAFLD have increased. In 2019, the population of the East Asia region had the highest Age-standardized DALY and mortality rates, followed by high-income Asia-Pacific and Central Asia populations. Although East Asia and high-income Asia-Pacific regions showed a decrease during the study period, Age-standardized DALY rates increased in Central Asia. High-income North American and Australasian populations also showed a significant increase in Age-standardized DALY.

Conclusions: Liver cancer remains an ongoing global threat. The burden of liver cancer associated with alcohol consumption and NASH/NAFLD is markedly increasing and projected to continuously increase. (Clin Mol Hepatol 2023;29:433-452)

Keywords: Global burden; Primary liver cancer; Incidence; Mortality; Aetiology

Corresponding author: Jae Il Shin
Department of Pediatrics, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2228-2050, Fax: +82-2-393-9118, E-mail: shinji@yuhs.ac
https://orcid.org/0000-0003-2326-1820

Seung Up Kim
Department of Internal Medicine, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2228-1944, Fax: +82-82-2-362-6884, E-mail: ksukorea@yuhs.ac
https://orcid.org/0000-0002-9658-8050

*These authors contributed equally to this work as co-first authors.

Editor: Dae Won Jun, Hanyang University College of Medicine, Korea
Received: Oct. 17, 2022 / Revised: Dec. 16, 2022 / Accepted: Dec. 27, 2022

Abbreviations:
GBD, Global Burden of Disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; SDI, sociodemographic index; SVR, sustained virological response; UI, uncertainty interval; DALY, disease-adjusted life year

INTRODUCTION

Primary liver cancer is a significant global health concern, representing the seventh most common type of cancer and the second leading cause of cancer-related deaths. The countries with the highest incidence of liver cancer mainly typically show lower levels of economic development, and most cases of liver cancer occur in geographically diverse countries, including those in North and West Africa (Gambia, Egypt, and Guinea) and East and Southeast Asia (Cambodia, Mongolia, and Vietnam). The major aetiologies of primary liver cancer include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD). However, the aetiologies greatly vary from region to region. More specifically, the most important risk factors in China and East Africa are chronic HBV infection and aflatoxin contamination in food, respectively, and in other countries such as Egypt and Japan, chronic HCV infection is often the main cause. In Western countries, chronic HCV infection, alcohol consumption, and obesity/diabetes are the most common causes of liver cancer.

The Global Burden of Disease (GBD) database was constructed to provide a tool for quantifying health losses from hundreds of diseases, injuries, and risk factors. The GBD was initiated and maintained by a consortium of more than 3,600 researchers in nearly 200 countries. The database comprises a comprehensive catalogue of surveys, censuses, vital statistics, and other health-related data such as death and disability. Therefore, the GBD database can provide helpful insights for understanding the nature of liver cancer, how the challenges are changing over time, and how to allocate health resources more effectively.

Because effective antivirals against chronic HBV and HCV infections have recently become available, the global distribution of the dominant aetiologies has changed. In particular, due to universal screening for HCV infection and direct-acting antivirals (DAAs) against chronic HCV infection, HCV is expected to be eliminated by 2030. However, trends in liver cancer based on specific aetiology and sociodemographic status have rarely been reported and compared.

In this study, we aimed to describe and analyse the updated temporal trends in aetiologies and sociodemographic status from 1990 to 2019 to provide insights into appropriate global intervention strategies.

MATERIALS AND METHODS

Overview

The GBD 2019 report consists of 369 diseases and injuries. Over 80 behavioural, environmental, occupational, and metabolic risk factors are also recorded. Estimation of the sampling error was described in detail in a previous study.

Data sources

Data on the number and Age-standardized rates of incidence and mortality of primary liver cancer were extracted from the GBD 2019 database (http://ghdx.healthdata.org/gbd-2019). Liver cancer data from 204 countries and territories were collected. This was further divided into five quintiles based on sociodemographic index (SDI; low, low-middle, middle, high-middle, high) and 21 geographic regions. Cancer incidence, mortality, and morbidity data used in the GBD 2019 included individual population-based cancer registries, Cancer Incidence in Five Continents (CI5), the Nordic Cancer Registries database and European Network of Cancer Registries. Primary liver cancer in the GBD 2019 report corresponds to C22–C22.8 and Z85.05 in the International Classification of Diseases 10th revision. Liver cancer cases were divided into five categories based on underlying aetiology (hepatitis B, hepatitis C, alcohol, non-alcoholic steatohepatitis [NASH]/NAFLD, and other causes) in the GBD 2019 database. The proportion of liver cancer cases based on aetiology was determined using a systematic literature search and various adjusting models (hepatitis B, hepatitis C, and NASH/NAFLD, and other causes) in the GBD 2019 database. The proportion of liver cancer cases based on aetiology was determined using a systematic literature search and various adjusting models (hepatitis B, hepatitis C, and NASH/NAFLD prevalence; alcohol consumption; hepatitis B vaccination coverage; and proportion of cirrhosis due to liver cancer subtyping). For every study, proportions of liver cancer due to the five aetiologies were calculated using five separate DisMod-Mr 2.1 models (aetiology split model). The estimated proportion was then used to split the total liver cancer estimates into aetiologies.

Sociodemographic index (SDI)

Socioeconomic development status was graded based on the SDI, which incorporates the total fertility rate in women <25 years of age, mean educational level for individuals ≥15 years of age, and lag-distributed income per person. The
method for generating the SDI has been described in detail in a previous report. The SDI values range between 0 and 1, which indicates the socioeconomic development level of a country on a scale of worst to best. SDI locations were chosen based on quintile of ranked SDI values. A specific country or region with SDI <20th, 20th–39th, 40th–59th, 60th–79th, and >80th percentage of the ranked SDI values was grouped as low, low-middle, middle, high-middle, and high SDI, respectively. The SDI quintiles were obtained from the GBD 2019 data and are presented in Supplementary Table 1.

Uncertainty analysis

The incidence and mortality rates in each year were assumed to follow a log-normal distribution, and the rates in different years were independent of each other. Based on these assumptions, 95% uncertainty indices (UIs) were calculated in each bootstrap draw based on the 25th and 975th ranked values across all 1,000 draws in the GBD 2019.

RESULTS

Global burden of liver cancer

A world map of Age-standardized disease-adjusted life year (DALY) rates (per 100,000 individuals) is shown in Figure 1A. At the global level, the number of DALYs from liver cancer increased from 11,278,630 (95% UI, 10,062,526–12,677,403) in 1990 to 12,528,422 (95% UI, 11,400,671–13,687,675) in 2019 (Table 1). The number of deaths also increased from 365,215 (95% UI, 329,967–405,774) to 484,577 (95% UI, 444,091–525,798) from 1990 to 2019 (Table 1), and the total number of liver cancer patients almost doubled from 433,327 (95% UI, 390,105–482,719) to 747,288 (95% UI, 680,904–822,765) during the same period (Supplementary Table 2).

Age-standardized DALY and mortality rates showed similar trends during the study period (Fig. 1B, C). From 1990 to 1996, Age-standardized DALY rate increased from 258.4 (95% UI, 230.9–290.1) to 284.1 (95% UI, 265–304.1), and Age-standardized mortality rate increased from 8.9 (95% UI, 8.1–9.9) to 10 (95% UI, 9.3–10.6). In the 1996 to 2012 period, decreasing age-standardized DALY and mortality rates were observed (in 2012, age-standardized DALY rate: 150.4, 95% UI, 143.4–158.5; age-standardized mortality rate: 5.9, 95% UI, 5.6–6.2). After 2012, age-standardized DALY and mortality rates slightly increased during the study period (in 2019, age-standardized-DALY rate: 151.1, 95% UI, 137.5–164.8; age-standardized mortality rate: 5.9, 95% UI, 5.4–6.4). Overall, both age-standardized DALY and mortality rates showed a decrease, with respective decreases of 41.5% (95% UI, 31.5–49.8) and 33.4% (95% UI, 23.2–41.9) during the study period. Age-standardized rates of prevalence and incidence showed a decline between 1990 and 2019: age-standardized prevalence rates slightly decreased from 10.2 (95% UI, 9.2–11.3) to 9.1 (95% UI, 8.3–10.0) and age-standardized incidence rates from 9 (95% UI, 8.1–10) to 6.5 (95% UI, 5.9–7.2; Supplementary Tables 2, 5).

The Republic of Korea shows an overall declining trend of liver cancer burden during the past decade, with age-standardized DALY rate decreasing 21.2% (95% UI, -31.2% to -10.6%; Fig. 2). In 1990, the Republic of Korea had a 390.8 (95% UI, 348.6–345.9) age-standardized DALY rate, 16.2 (95% UI, 14.5–17.9) age-standardized mortality rate, and 22.8 (95% UI, 18.7–27.3) age-standardized incidence rate for liver cancer (Fig. 2, Supplementary Figs. 25–27).

Burden of liver cancer based on geographic and sociodemographic region

The burden of liver cancer based on geographic and sociodemographic characteristics is summarized in Table 1. The age-standardized DALY rate of liver cancer varies widely worldwide. In 2019, among the 21 GBD-classified regions, East Asia had the highest age-standardized DALY rates (263.4; 95% UI, 221.3–312.2), followed by high-income populations in Asia-Pacific (238.6; 95% UI, 220.6–255.5) and Central Asia (213.5; 95% UI, 184.9–244.5). Although East Asia and high-income Asia-Pacific regions had the highest age-standardized incidence rates in 2019, Central Asia, high-income North America, and Australasia showed the largest percentage changes between 1990 and 2019. In 2019, the middle SDI quintile had the highest Age-standardized DALY, mortality, and incidence rates of 206.9, 7.9, and 8.3 (95% UI, 180.2–235, 95% UI, 7.0–8.9, and 95% UI, 7.2–
### Table 1. Age-standardized DALY rate, age-standardized mortality rate, and cases of liver cancer based on GBD and SDI at the regional level in 1990 and 2019

<table>
<thead>
<tr>
<th>Region</th>
<th>1990</th>
<th>2019</th>
<th>Percentage change in Age-standardized rates between 1990 and 2019</th>
<th>1990</th>
<th>2019</th>
<th>Percentage change in Age-standardized rates between 1990 and 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DALYs</td>
<td>Deaths</td>
<td>Counts (95% UI) Rate (95% UI)</td>
<td>Counts (95% UI) Rate (95% UI)</td>
<td>Counts (95% UI) Rate (95% UI)</td>
<td>Counts (95% UI) Rate (95% UI)</td>
</tr>
<tr>
<td>Global</td>
<td>11,278,630</td>
<td>365,215</td>
<td>258.4 (10,065,226, 12,677,403) 20.0 (230.9, 290.1)</td>
<td>12,528,422  151.1 (11,400,671, 13,687,675) 15.7 (137.5, 164.8)</td>
<td>-0.4 (329,967, 405,774) 8.9 (81.1, 9.9)</td>
<td>484,577 5.9 (-0.3)</td>
</tr>
<tr>
<td>High SDI</td>
<td>1,206,697</td>
<td>48,130</td>
<td>122.2 (1,173,184, 1,235,866) 12.8 (118.9, 125.3)</td>
<td>2,277,516 133.1 (2,140,287, 2,388,714) 12.7 (257.5, 139.3)</td>
<td>0.1 (46,467, 49,302) 4.7 (4.5, 4.8)</td>
<td>112,240 5.9 (0.3)</td>
</tr>
<tr>
<td>High-middle SDI</td>
<td>3,243,020</td>
<td>107,827</td>
<td>289.9 (2,790,860, 3,728,776) 21.2 (249.8, 332.6)</td>
<td>2,020,034 127.3 (2,214,723, 2,839,723) 11.8 (144.4)</td>
<td>-0.6 (94,484, 122,721) 10 (8.7, 11.3)</td>
<td>97,189 4.8 (-0.5)</td>
</tr>
<tr>
<td>Middle SDI</td>
<td>5,333,641</td>
<td>163,806</td>
<td>433.4 (4,608,989, 6,183,319) 37.6 (4,736,439, 6,221,172)</td>
<td>5,463,765 206.9 (180.2, 235) -0.5 (142,716, 189,464) 11.1 (13.1, 17.3)</td>
<td>163,806 15 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Low-middle SDI</td>
<td>1,139,257</td>
<td>34,779</td>
<td>154.5 (1,019,675, 1,268,092) 13.2 (139.2, 170.4)</td>
<td>1,616,488 109 (1,469,159, 1,785,003) 9.2 (99.2, 130.3)</td>
<td>-0.3 (31,450, 38,178) 5.6 (5.1, 6.1)</td>
<td>57,241 4.2 (-0.2)</td>
</tr>
<tr>
<td>Low SDI</td>
<td>353,051</td>
<td>10,563</td>
<td>113.1 (309,468, 398,851) 9.8 (98.8, 127.7)</td>
<td>663,784 101.2 (570,133, 758,108) 8.8 (88.4, 114.3)</td>
<td>0.1 (9,243, 11,898) 4.4 (3.8, 5.5)</td>
<td>20,756 3.9 (-0.1)</td>
</tr>
<tr>
<td>East Asia</td>
<td>7,723,663</td>
<td>237,005</td>
<td>755.4 (6,580,547, 9,129,280) 64.4 (6,479,839, 6,534,429)</td>
<td>5,941,179 263.4 (221,312, 3,112) -0.7 (202,341, 279,889) 22.9 (163,848, 228,758)</td>
<td>9.4 (7,8)</td>
<td></td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>551,810</td>
<td>17,574</td>
<td>180.3 (492,855, 604,067) 161.2 (943,489, 1,384,243)</td>
<td>1,149,098 177.5 (146,6, 213.5) 0 (15,676, 19,729) 6.7 (6.5)</td>
<td>42,862 7.3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Oceania</td>
<td>3,557</td>
<td>112</td>
<td>98.3 (3,003, 4,124) 82.6 (7,903, 8,495)</td>
<td>7,093 85.4 (713, 101.0) -0.1 (94, 131) 3.8 (3.2, 4.5)</td>
<td>233 3.5 (-0.1)</td>
<td></td>
</tr>
<tr>
<td>Central Asia</td>
<td>43,722</td>
<td>1,507</td>
<td>85.3 (38,393, 48,032) 75.7 (172,830, 213.5)</td>
<td>15,164 1.5 (148,859, 200,042) 1.2 (184,9, 244.5)</td>
<td>1.5 (1,348, 1,663) 3.2 (2.9, 3.6)</td>
<td>6,191 8.7 (1.7)</td>
</tr>
<tr>
<td>Central Europe</td>
<td>194,212</td>
<td>8,114</td>
<td>113.7 (188,478, 199,058) 127.7 (133,681, 182,107)</td>
<td>156,614 791 (677, 92.3) -0.4 (7,830, 8,322) 5.4 (5.5, 7.0)</td>
<td>7,202 3.4 (-0.4)</td>
<td></td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>115,457</td>
<td>4,224</td>
<td>43.5 (110,529, 121,300) 41.6 (234,701, 74.9)</td>
<td>709 74.9 (713, 101.0) 0.7 (4,224) 1.6 (4,224) 9.6 (2.9)</td>
<td>9,676 2.9 (0.9)</td>
<td></td>
</tr>
<tr>
<td>High-Income Asia</td>
<td>614,780</td>
<td>235,589</td>
<td>295.6 (594,699, 637,246) 285.8 (842,591, 983,716)</td>
<td>920,379 233.6 (205,032, 2,733,291) 65.3 (65,3, 86.6)</td>
<td>-0.3 (22,762, 24,374) 11.2 (15,1, 15.6)</td>
<td>49,685 10.8 (-0.1)</td>
</tr>
<tr>
<td>Pacific</td>
<td>51,484</td>
<td>464</td>
<td>50.3 (411,085, 11,938) 48.7 (43,655, 98.1)</td>
<td>43,655 98.1 (40,249, 47,404) 90.3 (90.3, 106.4)</td>
<td>0.9 (445, 481) 2 (1,9, 2.1)</td>
<td>2,006 4.1 (1.1)</td>
</tr>
<tr>
<td>Australasia</td>
<td>439,140</td>
<td>19,883</td>
<td>80.7 (426,685, 449,642) 78.5 (738,440, 836,208)</td>
<td>787,717 98.5 (93, 104.5) 0.2 (191,60, 204,19) 0.2 (3,3, 3.3)</td>
<td>3.4 (37,224, 42,876) 4.4 (4.1, 4.7)</td>
<td></td>
</tr>
<tr>
<td>Western Europe</td>
<td>18,439</td>
<td>755</td>
<td>39.1 (16,686, 20,246) 35.4 (43,354, 53.6)</td>
<td>43,354 53.6 (40,967, 46,273) 50.4 (50.4, 57)</td>
<td>0.4 (683, 829) 1.7 (1,5, 1)</td>
<td>2,027 2.4 (0.5)</td>
</tr>
<tr>
<td>Southern Latin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td>1990</td>
<td>2019</td>
<td>Percentage change in Age-standardized rates between 1990 and 2019</td>
<td>1990</td>
<td>2019</td>
<td>Percentage change in Age-standardized rates between 1990 and 2019</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Counts (95% UI)</td>
<td>Rate (95% UI)</td>
<td>Counts (95% UI)</td>
<td>Rate (95% UI)</td>
<td>Counts (95% UI)</td>
<td>Rate (95% UI)</td>
</tr>
<tr>
<td>High-income North America</td>
<td>167,210</td>
<td>51</td>
<td>608,194</td>
<td>105.5</td>
<td>1.1</td>
<td>0.8, 1.3</td>
</tr>
<tr>
<td>Caribbean</td>
<td>41,326</td>
<td>151.5</td>
<td>41,276</td>
<td>80.7</td>
<td>-0.5</td>
<td>6,778, 7,245</td>
</tr>
<tr>
<td>Andean Latin America</td>
<td>30,637</td>
<td>129.8</td>
<td>44,340</td>
<td>77.3</td>
<td>-0.4</td>
<td>946, 1,211</td>
</tr>
<tr>
<td>Central Latin America</td>
<td>84,956</td>
<td>89.2</td>
<td>197,475</td>
<td>82.8</td>
<td>-0.1</td>
<td>2,858, 3,233</td>
</tr>
<tr>
<td>Tropical Latin America</td>
<td>54,246</td>
<td>52.2</td>
<td>142,719</td>
<td>58.6</td>
<td>0.1</td>
<td>1,803, 1,949</td>
</tr>
<tr>
<td>North Africa and Middle East</td>
<td>319,736</td>
<td>161.1</td>
<td>731,622</td>
<td>153.3</td>
<td>0</td>
<td>8,575, 12,227</td>
</tr>
<tr>
<td>South Asia</td>
<td>512,899</td>
<td>72.9</td>
<td>1,085,515</td>
<td>71.3</td>
<td>0</td>
<td>15,854</td>
</tr>
<tr>
<td>Central Sub-Saharan Africa</td>
<td>28,800</td>
<td>77.3</td>
<td>51,448</td>
<td>65.3</td>
<td>-0.2</td>
<td>717</td>
</tr>
<tr>
<td>Eastern Sub-Saharan Africa</td>
<td>91,016</td>
<td>83</td>
<td>187,944</td>
<td>85.5</td>
<td>0</td>
<td>2,537</td>
</tr>
<tr>
<td>Southern Sub-Saharan Africa</td>
<td>59,594</td>
<td>181.7</td>
<td>122,195</td>
<td>188.8</td>
<td>0</td>
<td>2,076, 3,145</td>
</tr>
<tr>
<td>Western Sub-Saharan Africa</td>
<td>172,397</td>
<td>151.3</td>
<td>308,593</td>
<td>130.8</td>
<td>-0.1</td>
<td>5,308</td>
</tr>
<tr>
<td>Saharan Africa</td>
<td>145,973, 199,308</td>
<td>129.7, 176.3</td>
<td>252,949, 365,495</td>
<td>109.4, 152.3</td>
<td>-0.3, 0.1</td>
<td>4,553, 6,165</td>
</tr>
</tbody>
</table>

GBD, global burden of disease; SDI, sociodemographic index; UI, uncertainty index; DALY, disease-adjusted life year. Individual country data is described in Supplementary Tables 2-7.
9.5), respectively.

**Burden of liver cancer based on aetiology**

Globally, in 2019, HBV remained the leading cause of Age-standardized liver cancer DALY and mortality rates, followed by HCV, alcohol consumption, and NASH/NAFLD (Fig. 3). Although other aetiologies have remained stable or decreased during the past 10 years, the age-standardized DALY and mortality rates of liver cancer due to alcohol consumption and NASH/NAFLD have gradually increased over the past decade (Fig. 3). Data regarding liver cancer due to other causes is shown in Supplementary Figures 20-23.

**Burden of liver cancer due to HBV**

HBV accounts for the highest Age-standardized mortality rate of liver cancer. When stratified based on GBD region, noticeable decrease in Age-standardized DALY and mortality rates was observed in liver cancer caused by HBV in East Asia between 1990 and 2019 (-65.5%, 95% UI, -73.6% to -54% and -64.1%, 95% UI, -72.3% to -52.6%, respectively). However, East Asia remained the region with the highest age-standardized mortality rate due to HBV liver cancer. Central Asia, high-income North America, and Australasia experienced a steep increase in age-standardized DALY rate during the same period (129.9%, 95% UI, 89.5–177.7%; 81.7%, 95% UI,
### Table 2. Age-standardized DALY rate in 2019 and percentage change in age-standardized DALY rate between 1990 and 2019 of liver cancer caused by specific aetiology based on GBD region

<table>
<thead>
<tr>
<th>Region</th>
<th>Hepatitis B Age-standardized DALYs Rate in 2019 (95% UI)</th>
<th>Percentage change between 1990 and 2019</th>
<th>Percentage change between 1990 and 2019 (95% UI)</th>
<th>Alcohol Age-standardized DALYs Rate in 2019 (95% UI)</th>
<th>Percentage change between 1990 and 2019</th>
<th>NASH/NAFLD Age-standardized DALYs Rate in 2019 (95% UI)</th>
<th>Percentage change between 1990 and 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Asia</td>
<td>(69.1, 73.0)</td>
<td>-65.5</td>
<td>(72.9, -57.7)</td>
<td>(21.3, 27.5)</td>
<td>-60</td>
<td>(11.4, 14.4)</td>
<td>-57.7</td>
</tr>
<tr>
<td>(58.7, 80.6)</td>
<td>(-73.6, -54)</td>
<td></td>
<td></td>
<td>(9.2, 14.1)</td>
<td>(-66.1, -47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>(176.4, 215.6)</td>
<td>-12.2</td>
<td>(-21.5, 15.6)</td>
<td>(44.1, 58.4)</td>
<td>22.7</td>
<td>(15.6, 28.8)</td>
<td></td>
</tr>
<tr>
<td>(143.9, 205.6)</td>
<td>(-31.3, 10.6)</td>
<td></td>
<td></td>
<td>(11.2, 21.4)</td>
<td>(-21.6, 22.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oceania</td>
<td>73</td>
<td>-17.8</td>
<td>(11.6, 24.6)</td>
<td>(10.8, 11.8)</td>
<td>-3.5</td>
<td>(7.1, 3.0)</td>
<td></td>
</tr>
<tr>
<td>(55.5, 94.8)</td>
<td>(-34.2, 1.2)</td>
<td></td>
<td></td>
<td>(6.8, 16)</td>
<td>(23.5, 20.5)</td>
<td>(4.9, 10.2)</td>
<td>(-15.5, 27.2)</td>
</tr>
<tr>
<td>Central Asia</td>
<td>44.8</td>
<td>129.9</td>
<td>(45.6, 84)</td>
<td>(43.3, 48.4)</td>
<td>182.6</td>
<td>(14.6, 24.8)</td>
<td></td>
</tr>
<tr>
<td>(34.8, 57.7)</td>
<td>(89.5, 177.7)</td>
<td></td>
<td></td>
<td>(44.5, 84.4)</td>
<td>(134, 235.3)</td>
<td>(10.4, 20.5)</td>
<td>(188.1, 304.5)</td>
</tr>
<tr>
<td>Central Europe</td>
<td>60.6</td>
<td>-46.5</td>
<td>(12.1, 23.3)</td>
<td>(25.7, 41.6)</td>
<td>109.8</td>
<td>(5.9, 34.4)</td>
<td></td>
</tr>
<tr>
<td>(43.7, 80.9)</td>
<td>(-54.9, -37)</td>
<td></td>
<td></td>
<td>(25.7, 41.6)</td>
<td>(109.8, 203.9)</td>
<td>(4.7, 6.5)</td>
<td>(86.4, 136.6)</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>19.1</td>
<td>70.8</td>
<td>(13, 19)</td>
<td>(21.6, 32.6)</td>
<td>109.8</td>
<td>(5.4, 110.4)</td>
<td></td>
</tr>
<tr>
<td>(14.1, 25.9)</td>
<td>(48.1, 97.4)</td>
<td></td>
<td></td>
<td>(82.5, 143.5)</td>
<td>(19.7, 127.2)</td>
<td>(4.5, 6.5)</td>
<td>(86.4, 136.6)</td>
</tr>
<tr>
<td>High-income Asia</td>
<td>20.4</td>
<td>11.8</td>
<td>(89.8, 96.6)</td>
<td>(32.9, 41.3)</td>
<td>-14.4</td>
<td>(10, -9.4)</td>
<td></td>
</tr>
<tr>
<td>Pacific</td>
<td>(16.5, 25.2)</td>
<td>(-5.3, -30.1)</td>
<td></td>
<td>(25.6, 41.9)</td>
<td>-21.9</td>
<td>(4.1, 7.8)</td>
<td>(-44.3, -22.6)</td>
</tr>
<tr>
<td>(10.8, 20.4)</td>
<td>(57.8, 96.6)</td>
<td></td>
<td></td>
<td>(25.3, 31.8)</td>
<td>(-20.3, 2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australasia</td>
<td>88.6</td>
<td>73.2</td>
<td>(29.3, 46.8)</td>
<td>(38.3, 46.8)</td>
<td>83.6</td>
<td>(9.6, 175.7)</td>
<td></td>
</tr>
<tr>
<td>(75.8, 102.4)</td>
<td>(53, 93.8)</td>
<td></td>
<td></td>
<td>(64.1, 104.2)</td>
<td>(7.1, 12.6)</td>
<td>(135.8, 216.3)</td>
<td></td>
</tr>
<tr>
<td>Western Europe</td>
<td>16.3</td>
<td>15.2</td>
<td>(29, 43.6)</td>
<td>(35.9, 59.2)</td>
<td>26.4</td>
<td>(5.8, 47.4)</td>
<td></td>
</tr>
<tr>
<td>(12.1, 22.2)</td>
<td>(6.5, 25.6)</td>
<td></td>
<td></td>
<td>(28.7, 43.1)</td>
<td>(17.6, 36.4)</td>
<td>(36.1, 59.2)</td>
<td></td>
</tr>
<tr>
<td>Southern Latin</td>
<td>14.6</td>
<td>27.2</td>
<td>(19.2, 21.7)</td>
<td>(7.2, 17.2)</td>
<td>38.1</td>
<td>(6.7, 76.7)</td>
<td></td>
</tr>
<tr>
<td>(11.1, 19.9)</td>
<td>(11.9, 46)</td>
<td></td>
<td></td>
<td>(12.5, 22.1)</td>
<td>(21.2, 59.8)</td>
<td>(3.3, 10.6)</td>
<td>(54.2, 103.2)</td>
</tr>
<tr>
<td>High-income Asia</td>
<td>10.3</td>
<td>81.7</td>
<td>(29.3, 40.3)</td>
<td>(35.6, 123.7)</td>
<td>123.7</td>
<td>(9.8, 128.5)</td>
<td></td>
</tr>
<tr>
<td>America</td>
<td>(7.2, 14.4)</td>
<td>(58.4, 103.4)</td>
<td>(94.5, 136.9)</td>
<td>(29.2, 42.2)</td>
<td>(96.5, 151)</td>
<td>(8, 11.8)</td>
<td>(103.7, 148.8)</td>
</tr>
<tr>
<td>Caribbean</td>
<td>14.4</td>
<td>-46.6</td>
<td>(9.6, 21.4)</td>
<td>(26.5, 44.1)</td>
<td>44.1</td>
<td>(6.8, -40.5)</td>
<td></td>
</tr>
<tr>
<td>(117, 176)</td>
<td>(-59.3, -35.3)</td>
<td></td>
<td></td>
<td>(18.9, 36.1)</td>
<td>(-54.1, -31.6)</td>
<td>(47.9, 7.9)</td>
<td>(-50.7, -28.3)</td>
</tr>
<tr>
<td>Andean Latin</td>
<td>24.8</td>
<td>-46.1</td>
<td>(3.7, 17.7)</td>
<td>(15.4, 31.7)</td>
<td>-18.4</td>
<td>(7, -23)</td>
<td></td>
</tr>
<tr>
<td>America</td>
<td>(17.3, 34.1)</td>
<td>(-58.6, -31)</td>
<td>(74.9, -19.7)</td>
<td>(51.4, 31.7)</td>
<td>(-48.1, -19.7)</td>
<td>(48.9, 9.9)</td>
<td>(-40.9, -12.2)</td>
</tr>
<tr>
<td>Central Latin</td>
<td>34.5</td>
<td>-22.2</td>
<td>(29.3, 36.4)</td>
<td>(25.2, 32.6)</td>
<td>0.6</td>
<td>(7.3, 19.7)</td>
<td></td>
</tr>
<tr>
<td>America</td>
<td>(25.2, 44.9)</td>
<td>(-33.6, -7.5)</td>
<td>(20.4, 5.7)</td>
<td>(19.5, 31.9)</td>
<td>(13.6, 17.9)</td>
<td>(5.7, 39.4)</td>
<td>(2.9, 38.7)</td>
</tr>
<tr>
<td>Tropical Latin</td>
<td>13.4</td>
<td>-4.1</td>
<td>(179, 23.2)</td>
<td>(15.2, 20.1)</td>
<td>26.2</td>
<td>(3.6, 29.4)</td>
<td></td>
</tr>
<tr>
<td>America</td>
<td>(9.9, 18.2)</td>
<td>(-10.2, 3)</td>
<td>(9, 23.2)</td>
<td>(18.2, 35.3)</td>
<td>(18.2, 35.3)</td>
<td>(3.1, 4.2)</td>
<td>(21.7, 37.3)</td>
</tr>
<tr>
<td>North Africa and</td>
<td>11.8</td>
<td>-16.1</td>
<td>(50.5, 96.5)</td>
<td>(14.6, 4.8)</td>
<td>7.3</td>
<td>(15.2, 279)</td>
<td></td>
</tr>
<tr>
<td>Middle East</td>
<td>(10.1, 13.8)</td>
<td>(-32.6, 4.9)</td>
<td>(30.2, 38.3)</td>
<td>(9.3, 22.5)</td>
<td>(19.8, 40.5)</td>
<td>(10.5, 22.2)</td>
<td>(-2.9, 76.4)</td>
</tr>
</tbody>
</table>
Burden of liver cancer due to HCV

HCV is the second leading cause of liver cancer based on age-standardized DALY rates. Liver cancer due to HCV showed a different trend, with the high-income Asia-Pacific region showing the highest age-standardized DALY rate (98.2) in 2019 (Fig. 2D). In addition, DALY rates per 100,000 individuals based on age group increased steeply in younger ages and plateaued earlier for males (Fig. 4E).

Table 2. Continued

<table>
<thead>
<tr>
<th>Region</th>
<th>Hepatitis B</th>
<th>Hepatitis C</th>
<th>Alcohol</th>
<th>NASH/NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age-standardized DALYs Rate in 2019 (95% UI)</td>
<td>Percentage change between 1990 and 2019</td>
<td>Age-standardized DALYs Rate in 2019 (95% UI)</td>
<td>Percentage change between 1990 and 2019</td>
</tr>
<tr>
<td>Central Sub-Saharan Africa</td>
<td>24.3 (20.1, 29.3)</td>
<td>-16.6</td>
<td>26.7 (18.8, 33.3)</td>
<td>-12.8</td>
</tr>
<tr>
<td>Eastern Sub-Saharan Africa</td>
<td>15.9 (11.2, 21.1)</td>
<td>0.2</td>
<td>18.7 (13.9, 24.2)</td>
<td>4.2</td>
</tr>
<tr>
<td>Southern Sub-Saharan Africa</td>
<td>25.3 (18.5, 34.5)</td>
<td>-46.9 (55.3)</td>
<td>45.3 (36.6, 54.6)</td>
<td>5.0</td>
</tr>
<tr>
<td>Western Sub-Saharan Africa</td>
<td>76.7 (64.3, 91.5)</td>
<td>-35.4 (24.7)</td>
<td>24.1 (17.1, 31.5)</td>
<td>-17.2</td>
</tr>
</tbody>
</table>

GBD, global burden of disease; UI, uncertainty index; DALY, disease-adjusted life year.
showed an abrupt decline during the early 2000s, the high SDI region showed steep increase in age-standardized DALY and mortality rates in the 1990s but experienced a gradual decrease until 2019. Even after a gradual decrease, the high SDI quintile showed the highest age-standardized DALY and mortality rates (Fig. 5B, C). Notably, age-standardized DALY rates decreased for high and high-middle SDI quintiles during the last decade, while the other quintiles showed minimal to no change (Fig. 5B).

Figure 5D shows the sex differences in number of deaths and age-standardized mortality rates for liver cancer due to HCV infection. From 1990 to 2019, the age-standardized DALYs rate of liver cancer due to hepatitis HCV was higher in males than females. Age-standardized DALY rates showed a decreasing trend during the past decade in both sexes (Fig. 5D). The number of DALYs based on age group showed a unique pattern: in younger age groups, more DALYs occurred in males; however, after 85 years of age, this pattern was reversed (Fig. 5E).
Burden of liver cancer due to alcohol consumption

Liver cancer caused by alcohol consumption accounts for the third greatest aetiology in age-standardized DALY rates of liver cancer. For liver cancer due to alcohol consumption, Central Asia, high-income North America, and Eastern Europe experienced significant increase between 1990 and 2019, of 182.6% (95% UI, 134–235.3%), 125.7% (95% UI, 96.5–151%), and 109.8% (95% UI, 82.5–143.5%), respectively. At the country level, Mongolia had a significantly high age-standardized DALY rate of 786.6 per 100,000 individuals (95% UI, 516.1–1,130), followed by Gambia (201.5, 95% UI, 144.6–312) and Thailand (176.3, 95% UI, 112.8–265.3; Fig. 6A). Although liver cancer burden due to alcohol consumption decreased in the Republic of Korea by 4.9% (95% UI, -20% to -12.1%) from 2010 to 2019, the recent trend shows an increasing burden (in 2019, Age-standardized DALY rate: 65.8, 95% UI, 44.7–91.9;...
Middle and low-middle SDI quintiles showed an increasing age-standardized DALY rate trend, with the highest Age-standardized DALY rate in 2019 in the high SDI quintile (Fig. 6B, C).

Sex differences are prominent in liver cancer caused by alcohol consumption. Age-standardized DALY rates differed more than four-fold between males and females. The number of DALYs from alcohol-related liver cancer in males has significantly increased during the past decade (Fig. 6D). In addition, both the number of DALYs and DALY rates were higher in males than in females in each age group (Fig. 6E).

Figure 5. Global burden of liver cancer caused by hepatitis C virus (HCV). (A) World map of age-standardized disease-adjusted life year (DALY) rates (per 100,000 population) of liver cancer caused by HCV at the country level in 2019, (B) age-standardized DALY rates (per 100,000 population) of liver cancer caused by HCV at the sociodemographic index (SDI) regional level, (C) age-standardized mortality rates (per 100,000 population) of liver cancer caused by HCV at the SDI regional level (Age-standardized incidence rate data is shown in Supplementary Fig. 1), (D) number and age-standardized DALY rates (per 100,000 population) of liver cancer caused by HCV at the global level from 1990 to 2019 (number and age-standardized rate of incidence is shown in Supplementary Fig. 12), and (E) number and DALY rates (per 100,000 population) of liver cancer due to HCV at the global level based on age group in 2019 (number and Age-standardized rate of mortality is shown in Supplementary Fig. 13).
Burden of liver cancer due to NASH/NAFLD

In 2019, among the four major aetiologies of liver cancer, NASH/NAFLD showed the lowest age-standardized DALY rate. The highest percentage increase in age-standardized mortality rates of liver cancer due to NASH/NAFLD occurred in Central Asia (243.8%, 95% UI, 188.1–304.5%), Australasia (175.7%, 95% UI, 135.8–216.3%), and high-income North America (128.5%, 95% UI, 103.7–148.8%); Table 2). In 2019, Mongolia had the highest Age-standardized DALY rate of 167.2 (95% UI, 106.9–249) per 100,000 individuals, and Gambia and Guinea were the second and third highest countries (Fig. 7A). For the past decade, the Republic of Korea showed a decreased age-standardized DALY rate of 14.8% (95% UI, -27% to -0.6%) with recent years plateauing (in 2019, Age-standardized DALY rate: 18.4, 95% UI, 12.7–26.8; Age-stan-
In 2019, the middle SDI quintile had the highest age-standardized DALY and mortality rates, which have been increasing during the past decade. Unlike the comparatively low age-standardized DALY and mortality rates, the age-standardized incidence rates were relatively high in the high SDI quintile compared with the middle SDI quintile (Fig. 7B, C, Supplementary Fig. 17).

The number of DALYs from NASH/NAFLD-related liver cancer has increased for both sexes during the past decade (Fig. 7D). In addition, after the 75–79-year age group, females showed a higher number of DALYs but with a similar pattern in rate compared with males (Fig. 7E).
DISCUSSION

In the present study, we showed that the crude numbers of DALYS, deaths, and incident cases of liver cancer significantly increased between 1990 and 2019. However, age-standardized DALYS, mortality, and incidence rates have declined. The discrepancy between the number and rates of DALYS, deaths, and cases might be explained by the aging population structure.<ref>10 With continued aging and population growth, the number and rate of incidence, as well as years of life lost and mortality, are projected to increase in the near future.<ref>11-13

Concordant with previous studies, HBV remains the leading cause of liver cancer mortality and incidence, followed by HCV, alcohol consumption, and NASH/NAFLD.<ref>14 Age-standardized mortality rates of liver cancer due to HBV and HCV have decreased during the past decade, possibly due to the effect of HBV vaccination and nucleos(t)ide analogues (NUCs)<ref>15 and novel DAAs against HCV.<ref>16,17 However, age-standardized mortality rates of liver cancer due to alcohol consumption and NASH/NAFLD have increased.

Patterns of liver cancer burden also vary significantly between regions and countries. In 2019, the liver cancer burden due to HBV was high in HBV-endemic regions such as East Asia, high-income Asia-Pacific, Southeast Asia, and Southern and Western Sub-Saharan Africa.<ref>18 However, most of these regions are experiencing or will have a significant decrease in the burden of liver cancer due to HBV through universal HBV immunization programs and HBV screenings.<ref>18-20 In contrast, the overall age-standardized mortality and incidence rates were low in the high-income countries of North America and Australasia, but increased to >80% in 2019 compared with 1990. In addition, the GBD 2019 data showed an inverse trend in the age-standardized incidence rates of acute HBV infection, cirrhosis, and other chronic diseases due to HBV compared with HBV-attributable liver cancer in these regions (Supplementary Figs. 1–6). Therefore, despite the current use of potent NUCs, liver cancer progression rates are not well controlled.<ref>21-24 In the era of pre-NUCs, patients with chronic HBV infection would die before de novo hepatocarcinogenesis due to hepatic decompensation. Because HCC risk cannot be eliminated using only potent NUCs, the development of novel treatments is required to effectively eradicate closed circular DNA in hepatocytes. Furthermore, a more effective HCC surveillance strategy should be established to detect patients at an early stage and implement curative treatment.

In contrast to HBV infection, liver cancer due to HCV infection has a different threat pattern. Countries with high SDI were more affected by liver cancer due to HCV infection, with the high-income Asia-Pacific region showing the highest age-standardized mortality rates. However, with the development of DAAs with sustained virological response rates near 95%, the incidence and mortality caused by liver cancer due to HCV are expected to significantly decrease in the next decades.<ref>25-30 Notably, in high-income North America and Australasia, the age-standardized mortality rates have doubled in 2019 compared with 1990. This trend, however, is likely due to the 20- to 40-year lag from HCV infection to HCC progression.<ref>31 The effect of the 1945–1965 birth cohort in the United States with significantly high HCV infection rates<ref>22,23 and the use of injectable drugs in Australia<ref>32 could be causing this increase. Thus, even with careful measures, the burden of liver cancer due to HCV infection is likely to increase before the effects of intervention occur. In contrast to the past decade, when mainly high and high-middle SDI quintiles showed a decrease in age-standardized DALY rates, low- and middle-income countries are also likely to benefit from DAAs because of the production of cost-effective generic DAAs in the near future.<ref>35,36 Only 21% of the HCV-infected population knew their diagnosis and only 62% were treated with DAAs in 2019 according to a WHO report,<ref>37 with many low- and middle-income countries lacking current standard diagnostic methods.<ref>38 Thus, systematic and universal screening and appropriate referrals for HCV in endemic regions should be recommended with development of novel cost-effective diagnostic tests.<ref>38-41 However, to prevent infection, an effective HCV vaccine is needed.<ref>42,43

Contrary to liver cancer due to HBV and HCV, the age-standardised incidence and mortality of liver cancer due to alcohol consumption are increasing. Assuming an 8- to 9-year latency period of alcohol-induced liver cancer,<ref>44,45 the increasing trend of liver cancer due to alcohol consumption is concordant with the WHO Global information system on alcohol and health data (alcohol, total per capita [15+] consumption). A sharp increase was observed from 5.3 litres (95% confidence interval [CI] 5–6.5) in 2005 to 6.1 litres (95% CI 5.8–6.5) in 2010.<ref>46 If the trend of liver cancer due to alcohol consumption follows the alcohol consumption data, the number of liver cancers due to alcohol consumption is likely to remain steady in the next decade as alcohol consumption remains steady or possibly decreases (2019 alcohol consump-
Sex and age differences were observed in liver cancer burden. Males tended to have a higher burden of liver cancer due to all major aetiologies, except NASH/NAFLD. The HBV genome has been suggested to interact with androgens, leading to increased expression of oncoproteins. Oestrogen receptor expression and epigenetic mechanisms are associated with HCV-HCC, and gender differences exist in alcohol consumption. Various mechanisms have been proposed, including the protective role of oestrogen, HCC-promoting role of androgens, and additional role of sex chromosomes. Furthermore, the age group with the highest incidence in females was older and postmenopausal, which may be partially explained by the progression of liver fibrosis that can accelerate after menopause, and because postmenopausal hormone therapy has a protective effect against liver cancer.

The main strength of the present study includes the analysis of the most recent data on the global burden of liver cancer, focusing on DALYs of major liver cancer aetiologies. To the best of our knowledge, this study is the first to analyse in-depth DALYs of liver cancer based on aetiology. However, this study had several limitations. First, because data from the GBD 2019 database were analysed, the same limitations of the original dataset were applicable to the current study. Major limitations include the availability of primary data and the use of statistical modelling to generate estimates. For regions that lacked sufficient quality data, GBD estimates relied on predictive covariates and modelling measures. In addition, even when primary data were available, delay in data reporting and data obtained without using the preferred case definition could limit accuracy of the estimates. Thus, results need to be carefully interpreted regarding uncertainty intervals. In addition, due to the lack of data, various types of cancer have been widely identified as liver cancer, which hampered accurate analysis of gender and age differences. Third, the GBD 2019 database lacks detailed information other than epidemiological data such as vaccination policy changes, antiviral therapy use, and public health measures. Thus, this detailed information could not be included in the analysis.

In conclusion, despite substantial advances in prevention, diagnosis, and treatment, liver cancer continues to be an ongoing global threat. In particular, the liver cancer burden due to alcohol consumption and NASH/NAFLD is increasing and will continue to significantly increase if an effective intervention is not implemented. Because the burden of liver cancer significantly varies based on geographic region, SCI, sex, and age group, a more tailored approach should be implemented.
Lay summary

The global distribution of dominant liver cancer etiologies likely differs across global regions due to differential distribution or availability of effective antiviral drugs. Updated temporal trends of liver cancer aetiologies and sociodemographic status from 1990 to 2019 based on the Global Burden of Disease 2019 report were analysed in this study.

Authors’ contribution
S Choi, BK Kim, DK Yon, SW Lee, JI Shin, and SU Kim: study concept and design; S Choi and HG Lee: data acquisition and analysis; S Choi, BK Kim, JI Shin and SU Kim: manuscript drafting and data interpretation; S Choi, BK Kim, DK Yon, SW Lee, HG Lee, HH Chang, JI Shin, SU Kim, S Park, A Koyanagi, L Smith, L Jacob, E Dragiotiv, and J Radua: manuscript revision, provided critical comments, and wrote the final version of the manuscript; BK Kim, DK Yon, SW Lee, JI Shin, and SU Kim: study supervision. All authors made substantial contributions to the following: (1) conception and design of the study, data acquisition, or analysis and interpretation of data; (2) drafting or critical revision of the article for intellectual content; and (3) final approval of version to be submitted.

Acknowledgements
This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2019R1A2C4070136) and by the Technology Innovation Program (20013712) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This research was also funded by the National Research Foundation of Korea (NRF-2021 R1I1A2059735).

Conflicts of Interest
Seung Up Kim has served as an advisory committee member Gilead Sciences, Bayer, Eisai, and Novo Nordisk. He is a speaker for Gilead Sciences, GSK, Bayer, Eisai, Abbvie, EchoSens, MSD, Eisai, Otsuka, and Bristol-Myers Squibb. He has also received a research grant from Abbvie and Bristol-Myers Squibb. The other authors declare that they have no competing interests.

SUPPLEMENTARY MATERIAL

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

REFERENCES

9. Global Burden of Disease Liver Cancer Collaboration; Akinyem-


2020;26:383-400.
Factors associated with unrecognized cirrhosis in patients with hepatocellular carcinoma

Yi-Te Lee¹, Mohammad A. Karim², Hye Chung Kum², Sulki Park², Nicole E. Rich³, Mazen Noureddin⁴,⁵, Amit G. Singal³, and Ju Dong Yang⁴,⁵,⁶

¹California NanoSystems Institute, Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA; ²Population Informatics Lab, School of Public Health, Texas A&M University, College Station, TX; ³Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center, Dallas, TX; ⁴Karsh Division of Gastroenterology and Hepatology, ⁵Comprehensive Transplant Center, ⁶Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Study Highlights

- Cirrhosis is the most important risk factor of HCC; however, early cirrhosis is often undiagnosed. We characterized factors associated with unrecognized cirrhosis in a national sample of HCC patients from the United States. Among HCC patients with cirrhosis, 57.4% had unrecognized cirrhosis, with the highest proportion (76.3%) among those with NAFLD-related HCC. Male sex (aOR: 2.12, 95% CI: 1.83–2.46), non-Hispanic Black race (aOR: 1.93, 95% CI: 1.45–2.57), and NAFLD etiology (aOR: 4.46, 95% CI: 3.68–5.41) were associated with having unrecognized cirrhosis. Unrecognized cirrhosis was associated with worse overall survival compared to recognized cirrhosis. Our findings suggest these groups as important intervention targets to improve HCC surveillance uptake.
INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer worldwide. Cirrhosis caused by any etiology is the most important risk factor of HCC, and over 90% of patients with HCC in the Western world have underlying cirrhosis. Professional society guidelines recommend patients with cirrhosis undergo semiannual surveillance for HCC. A systematic review of cohort studies highlighted a consistent association between HCC surveillance and improved clinical outcomes, including early tumor detection and HCC mortality.

However, HCC surveillance is underused in clinical practice related to patient- and provider-level barriers. Although there are failures at multiple steps in the cancer screening continuum, one common failure is under-recognition of cirrhosis. Early compensated cirrhosis is often asymptomatic and can be undiagnosed for years, particularly given a lack of systematic screening for cirrhosis. Patients with unrecognized cirrhosis are not enrolled in HCC surveillance programs so can experience late-stage HCC detection and worse survival.

Background/Aims: Cirrhosis is the most important risk factor of hepatocellular carcinoma (HCC), and patients with cirrhosis are recommended to receive semiannual surveillance for early HCC detection. However, early cirrhosis is often asymptomatic and can go undiagnosed for years, leading to underuse of HCC surveillance in clinical practice. We characterized the frequency and associated factors of unrecognized cirrhosis in a national sample of patients with HCC from the United States.

Methods: HCC patients aged 68 years and older, diagnosed during 2011 to 2015 were included from the SEER-Medicare Linked Database. If cirrhosis was diagnosed within 6 months immediately preceding HCC diagnosis or after HCC diagnosis, cases were categorized as unrecognized cirrhosis. Factors associated with unrecognized cirrhosis were identified using logistic regression analyses. Factors associated with overall survival were evaluated using Cox regression analyses.

Results: Among 5,098 HCC patients, 74.8% patients had cirrhosis. Among those with cirrhosis, 57.4% had unrecognized cirrhosis, with the highest proportion (76.3%) among those with NAFLD-related HCC. Male sex (aOR: 2.12, 95% CI: 1.83–2.46), non-Hispanic Black race (aOR: 1.93, 95% CI: 1.45–2.57), and NAFLD etiology (aOR: 4.46, 95% CI: 3.68–5.41) were associated with having unrecognized cirrhosis. Among NAFLD-related HCC patients, male sex (aOR: 2.32, 95% CI: 1.71–3.14) was associated with unrecognized cirrhosis. Unrecognized cirrhosis was independently associated with worse overall survival (aHR: 1.17, 95% CI: 1.08–1.27) compared to recognized cirrhosis.

Conclusions: Unrecognized cirrhosis is common in NAFLD-related HCC, particularly among male and Black patients, highlighting these groups as important intervention targets to improve HCC surveillance uptake and outcomes. (Clin Mol Hepatol 2023;29:453-464)

Keywords: Liver cirrhosis; NAFLD; Hepatocellular carcinoma; Cancer screening

Corresponding author: Ju Dong Yang
Karsh Division of Gastroenterology and Hepatology, Comprehensive Transplant Center, Samuel Oschin Comprehensive Cancer Institute, 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: Donghee Kim, Stanford University School of Medicine, CA, USA
Received: Dec. 15, 2022 / Revised: Jan. 15, 2023 / Accepted: Jan. 29, 2023

Abbreviations:
aHR, adjusted hazard ratio; ALD, alcoholic liver disease; aOR, adjusted odds ratio; API, Asian/Pacific Islander; CI, confidence interval; FIB-4, Fibrosis-4; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; ICD-O-3, International Classification of Diseases for Oncology, 3rd edition; Metro, metropolitan; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; OS, overall survival; SEER, Surveillance, Epidemiology, and End Results; US, United States

https://doi.org/10.3350/cmh.2022.0450
Identifying factors associated with under-recognition of cirrhosis can help target intervention strategies, such as increased use of non-invasive markers of fibrosis. A prior retrospective cohort study from the Veterans Administration (VA) reported nonalcoholic fatty liver disease (NAFLD) is associated with a 4.8-fold increased risk of unrecognized cirrhosis. However, it is unclear if these data are generalizable to non-VA populations given differences in patient populations as well as practice patterns.

Herein, we aimed to characterize the frequency and associated factors of unrecognized cirrhosis and its impact on overall survival in a national sample of patients with HCC from the United States.

MATERIALS AND METHODS

Study population

HCC patient data were extracted from the SEER-Medicare Linked Database, which linked data on incident cancer cases from SEER program’s 18 cancer registries to data from Medicare, the primary health insurer for individuals aged 65 years and older. The inclusion criteria for HCC cases were (1) International Classification of Diseases (ICD)-Oncology-3 codes, site: C22.0 AND histology: 8170–8175, AND (2) adults ≥68 years of age, AND (3) HCC was diagnosed between 2011 and 2015. The reason for limiting the study cohort to adults ≥68 years of age is to ensure a 3-year follow-up period after Medicare enrollment for identifying potential risk factors and etiologies for HCC. The exclusion criteria (Supplementary Fig. 1) were (1) HCC cases diagnosed only based on death certificate without microscopic confirmation (n=437), OR (2) HCC cases from Medicare Part A and B enrollment with fewer than 3-year follow-up after Medicare enrollment, OR HCC cases with fewer than 6-month follow-up after HCC diagnosis (n=1,886), OR (3) HCC cases from Medicare health maintenance organizations (HMOs) enrollment (n=3,487). We excluded HCC cases from Medicare HMOs because Medicare HMOs plans were not required to submit individual claims information to the Centers for Medicare and Medicaid Services, which might introduce bias to subsequent analyses.

Cirrhosis was defined based on ICD-9 or 10 codes from Medicare claims, or presence of cirrhosis complications including ascites, hepatic encephalopathy, esophageal varices, etc (Supplementary Table 1). If cirrhosis was diagnosed within 6 months immediately preceding HCC diagnosis, or after HCC diagnosis, cases were categorized as “unrecognized cirrhosis.” Etiology of HCC was defined based on ICD-9 or 10 codes.

For overall survival (OS) analyses, we excluded HCC cases without follow-up after diagnosis OR HCC cases who died at the same calendar month of HCC diagnosis (i.e., 0-month follow-up, Supplementary Fig. 1) (n=710).

Study variables

Demographic and clinical variables including sex, age, race/ethnicity (categorized as non-Hispanic White, non-Hispanic Black, non-Hispanic Asian/Pacific Islander [API]/Others, and Hispanic), regions stratified by poverty level (as determined by United States Census data), rural-urban regions (level of metropolitan and nonmetropolitan based on the United States Department of Agriculture Economic Research Service definition), National Cancer Institute (NCI) Comorbidity Index, liver disease etiology, and the presence of diabetes, ascites, and hepatic encephalopathy were included in this study.

To calculate the NCI Comorbidity Index for representing the noncancer comorbidity, we applied the ICD diagnosis and procedure codes one year before HCC diagnosis. To avoid collinearity in the subsequent multivariable analyses, the NCI Comorbidity Index for HCC patients was calculated after excluding liver disease and diabetes. To determine the etiology of HCC, Medicare claims ICD-9 or 10 codes for hepatitis C virus (HCV), hepatitis B virus (HBV), alcoholic liver disease (ALD), NAFLD, and others were used. We classified patients with ICD-9 or 10 code for obesity, diabetes, history of bariatric surgery or both dyslipidemia and hypertension in the absence of HBV, HCV, alcohol abuse, and other known liver disease as NAFLD since NAFLD is often under-coded.

For patients with ≥ two etiologies, the etiology was assigned by the following hierarchy: HCV>HBV>ALD>others>NAFLD. The ICD-9 or 10 codes for defining chronic liver diseases, diabetes, and complications related to cirrhosis were provided in Supplementary Table 2 and Supplementary Table 3, respectively.

The variables of tumor stage and treatment type were included in the OS analyses. We defined early-stage HCC as a single tumor, less than or equal to 5 cm in diameter without vascular invasion or extrahepatic metastasis since SEER-Medicare Linked Database only categorizes tumor number as
unifocal or multifocal. Treatments were stratified as potentially curative treatments (liver resection, liver transplantation, and tumor ablation) and non-curative treatments (chemoembolization, radioembolization, other radiation, systemic treatment, and others/best supportive care).

Table 1. Clinical characteristics of HCC patients with recognized cirrhosis, unrecognized cirrhosis, or no cirrhosis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Recognized cirrhosis (n=1,625)</th>
<th>Unrecognized cirrhosis (n=2,190)</th>
<th>No cirrhosis (n=1,283)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex</td>
<td>962 (59.2)</td>
<td>1,569 (71.6)</td>
<td>907 (70.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>75.2±5.67</td>
<td>76.8±6.11</td>
<td>78.6±6.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>968 (59.6)</td>
<td>1,378 (63.0)</td>
<td>878 (68.4)</td>
<td>-</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>100 (6.1)</td>
<td>180 (8.2)</td>
<td>110 (8.6)</td>
<td>-</td>
</tr>
<tr>
<td>Non-Hispanic API/Others</td>
<td>257 (15.8)</td>
<td>360 (16.4)</td>
<td>193 (15.0)</td>
<td>-</td>
</tr>
<tr>
<td>Hispanic</td>
<td>300 (18.5)</td>
<td>272 (12.4)</td>
<td>102 (8.0)</td>
<td>-</td>
</tr>
<tr>
<td>Poverty level</td>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>0% to &lt;5% poverty</td>
<td>318 (19.6)</td>
<td>447 (20.4)</td>
<td>246 (19.2)</td>
<td>-</td>
</tr>
<tr>
<td>5% to &lt;10% poverty</td>
<td>363 (22.3)</td>
<td>520 (23.7)</td>
<td>309 (24.1)</td>
<td>-</td>
</tr>
<tr>
<td>10% to &lt;20% poverty</td>
<td>517 (31.8)</td>
<td>659 (30.1)</td>
<td>384 (29.9)</td>
<td>-</td>
</tr>
<tr>
<td>20% to 100% poverty</td>
<td>427 (26.3)</td>
<td>564 (25.8)</td>
<td>344 (26.8)</td>
<td>-</td>
</tr>
<tr>
<td>Rural-Urban</td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Metro &gt;1 million</td>
<td>986 (60.7)</td>
<td>1,333 (60.9)</td>
<td>710 (55.3)</td>
<td>-</td>
</tr>
<tr>
<td>Metro 250k to1 million</td>
<td>340 (20.9)</td>
<td>408 (18.6)</td>
<td>280 (21.8)</td>
<td>-</td>
</tr>
<tr>
<td>Metro &lt;250k</td>
<td>132 (8.1)</td>
<td>162 (7.4)</td>
<td>120 (9.4)</td>
<td>-</td>
</tr>
<tr>
<td>Non-Metro/Rural</td>
<td>167 (10.3)</td>
<td>287 (13.1)</td>
<td>173 (13.5)</td>
<td>-</td>
</tr>
<tr>
<td>NCI Comorbidity Index</td>
<td></td>
<td></td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>Low (0 to 2)</td>
<td>1,223 (75.3)</td>
<td>1,660 (75.8)</td>
<td>959 (74.7)</td>
<td>-</td>
</tr>
<tr>
<td>Moderate (&gt;2 to 4)</td>
<td>221 (13.6)</td>
<td>312 (14.2)</td>
<td>192 (15.0)</td>
<td>-</td>
</tr>
<tr>
<td>High (&gt;4)</td>
<td>181 (11.1)</td>
<td>218 (10.0)</td>
<td>132 (10.3)</td>
<td>-</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV</td>
<td>834 (51.3)</td>
<td>692 (31.6)</td>
<td>189 (14.7)</td>
<td>-</td>
</tr>
<tr>
<td>NAFLD</td>
<td>249 (15.3)</td>
<td>801 (36.6)</td>
<td>763 (59.5)</td>
<td>-</td>
</tr>
<tr>
<td>ALD</td>
<td>395 (24.3)</td>
<td>414 (18.9)</td>
<td>86 (6.7)</td>
<td>-</td>
</tr>
<tr>
<td>HBV</td>
<td>79 (4.9)</td>
<td>120 (5.5)</td>
<td>47 (3.7)</td>
<td>-</td>
</tr>
<tr>
<td>Other/None</td>
<td>68 (4.2)</td>
<td>163 (7.4)</td>
<td>198 (15.4)</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1,083 (66.6)</td>
<td>1,408 (64.3)</td>
<td>771 (60.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascites</td>
<td>986 (60.7)</td>
<td>1,227 (56.0)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td>540 (33.2)</td>
<td>335 (15.3)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Early stage*</td>
<td>485 (29.9)</td>
<td>301 (13.7)</td>
<td>181 (14.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Curative treatment†</td>
<td>474 (29.2)</td>
<td>435 (19.9)</td>
<td>258 (20.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean±standard deviation.

ALD, alcoholic liver disease; API, Asian/Pacific Islander; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; Metro, metropolitan; NAFLD, nonalcoholic fatty liver disease; NCI, National Cancer Institute.

*Early-stage HCC was defined as a single tumor, less than or equal to 5 cm in diameter without vascular invasion or extrahepatic. †Curative treatments were defined as liver resection, liver transplantation, and tumor ablation.
Statistical analysis

Demographic and clinical variables among patients with unrecognized cirrhosis, recognized cirrhosis, and no cirrhosis were compared using chi-square test for categorical variables and one-way analysis of variance test for continuous variable (i.e., age). Factors associated with unrecognized cirrhosis were identified using univariate and multivariable logistic regression analyses. OS probabilities of HCC patients stratified by cirrhosis status were estimated using the Kaplan-Meier method and compared using the log-rank test. Factors associated with OS were evaluated using univariate and multivariable Cox regression analyses.

All statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) and Stata 16.1 (StataCorp, College Station, TX, USA) software with two-sided tests and a significance level of 0.05.

RESULTS

Clinical characteristics

We identified 5,098 eligible patients with HCC, of whom NAFLD (35.6%) was the leading underlying etiology, followed by HCV (33.6%), ALD (17.6%), other/no etiologies (8.4%), and HBV (4.8%). A total of 3,815 patients with HCC (74.8%) were diagnosed with underlying cirrhosis, although there was a wide variation between etiologies (89.0% HCV, 80.9% HBV, 90.4% ALD, 57.9% NAFLD, and 53.8% other/no etiologies).

Among patients with cirrhosis, 2,190 patients (57.4%) had unrecognized cirrhosis. The clinical characteristics of HCC patients with recognized cirrhosis, unrecognized cirrhosis, and no cirrhosis were summarized in Table 1. Compared to those with recognized cirrhosis, a higher proportion of patients with unrecognized cirrhosis were older, male, non-Hispanic Black, and had underlying NAFLD. In fact, 76.3% of patients NAFLD and 70.6% of those with other etiologies had unrecognized cirrhosis at HCC diagnosis (Fig. 1).

Factors associated with unrecognized cirrhosis

Among HCC patients with cirrhosis, univariate logistic regression analysis revealed older age, male sex, living in non-metro/rural counties, and NAFLD etiology as being associated with greater odds of having unrecognized cirrhosis, while Hispanic ethnicity and higher NCI comorbidity index were associated with lower odds of unrecognized cirrhosis (Table

![Figure 1. Proportion of HCC patients with unrecognized cirrhosis by etiology. A high proportion of individuals with NAFLD-related HCC had unrecognized cirrhosis. ALD, alcoholic liver disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease.](#)
In multivariable logistic regression analysis, older age (adjusted odds ratio [aOR]: 1.04, 95% confidence interval [CI]: 1.03–1.05), male sex (aOR: 2.12, 95% CI: 1.83–2.46), non-Hispanic Black race (reference: non-Hispanic White; aOR: 1.93, 95% CI: 1.45–2.57), and NAFLD etiology (reference: HCV; aOR: 4.46, 95% CI: 3.68–5.41) were associated with unrecognized cirrhosis (Table 2). Conversely, Hispanic ethnicity (aOR: 0.79, 95% CI: 0.65–0.96), higher NCI comorbidity index (aOR: 0.72, 95% CI: 0.57–0.90), and diabetes (aOR: 0.73, 95% CI: 0.63–0.85) were inversely associated with having unrecognized cirrhosis in multivariable analysis (Table 2).

Subgroup analysis was performed to further identify factors associated with unrecognized cirrhosis among cirrhotic patients with NAFLD-related HCC. Male sex (aOR: 2.32, 95% CI: 1.71–3.14) and older age (aOR: 1.03, 95% CI: 1.00–1.05) was associated with unrecognized cirrhosis, while higher NCI comorbidity index (aOR: 0.58, 95% CI: 0.39–0.86) was inversely associated (Table 3).
Association between unrecognized cirrhosis and overall survival

Compared to patients with recognized cirrhosis, significant lower proportion of patients with unrecognized cirrhosis were presented with early-stage HCC (13.7% vs. 29.9%) and underwent potentially curative treatments (19.9% vs. 29.2%) (Table 1). Median OS were 20 months (interquartile range [IQR]: 9–43 months), 12 months (IQR: 4–33 months), and 15 months (IQR: 6–41 months) for patients with recognized cirrhosis, unrecognized cirrhosis, and no cirrhosis, respectively ($P<0.001$; Fig. 2). In multivariable Cox regression analysis adjusting for the demographic and clinical variables, tumor stage, and treatment type (Table 4), unrecognized cirrhosis was independently associated with worse OS (adjusted HR [aHR]: 1.17, 95% CI: 1.08–1.27) compared to recognized cirrhosis, while patients with no cirrhosis had favorable OS (aHR: 0.84, 95% CI: 0.76–0.93).

DISCUSSION

We investigated the frequency and associated factors for unrecognized cirrhosis among patients with cirrhosis and HCC using a large United States Medicare-based database. We found over 50% of HCC patients had unrecognized cirrhosis prior to HCC diagnosis, including three-fourths of those with NAFLD-related HCC. Older age, male sex, Non-Hispanic Black race, and NAFLD etiology were independently associated with greater odds of unrecognized cirrhosis, while Hispanic ethnicity, higher comorbidity, and diabetes were associated with lower odds of having unrecognized cirrhosis.
Lastly, our study confirmed that HCC patients with unrecognized cirrhosis were associated with worse survival than those with recognized cirrhosis.

In line with the findings in the previous VA study, we showed that NAFLD was associated with a more than fourfold increased likelihood of having unrecognized cirrhosis compared to HCV. A recent study showed that only 4.4% of patients with NAFLD in the United States were aware of their liver disease, much lower than the percentages of patients with HCV (42.4%) and HBV (17.2%). The low awareness of liver diseases among patients with NAFLD could be attributed to the insufficient understanding of NAFLD for both the general population and non–hepatologist physicians. The increasing burden of NAFLD in the world, American Association of Clinical Endocrinology and American Association for the Study of Liver Diseases recently provided a clinical practice guideline for diagnosis and management of NAFLD to prevent the development of liver cirrhosis and related comorbidities. The guideline suggests using liver fibrosis prediction calculations (e.g., Fibrosis-4) to initially assess the risk of advanced liver fibrosis in NAFLD patients, and triage the high-risk patients for fibrosis/cirrhosis screening by transient elastography. With increased availability of non-invasive biomarkers of fibrosis and transient elastography, the guideline will potentially increase recognition of NAFLD-related cirrhosis, especially for primary care physicians and endocrinologists who often look after NAFLD patients.

In overall and NAFLD subgroup analyses, we observed sex differences with the male having more than a two-fold increased likelihood of having unrecognized cirrhosis, which could arise from the variance of adherence to regular follow-up with physician and screening test. For example, a United States cohort study demonstrated females were more compliant with HCC surveillance than males. More recently, a study exploring potential reasons for HCC screening underuse showed similar results, indicating that men were less likely to stick to regular outpatient care than women. Even patients having regular outpatient care, failure of receiving ultrasound or noninvasive biomarkers for fibrosis might cause unrecognition of cirrhosis. Further investigation is warranted to understand underlying causes of this sex difference.

We noted Non-Hispanic Black race was associated with increased likelihood of having unrecognized cirrhosis. Higher likelihood of having unrecognized cirrhosis in non-Hispanic

![Figure 2](https://doi.org/10.3350/cmh.2022.0450)
Table 4. Factors associated with overall survival among HCC patients (n=4,388)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Univariate analysis</th>
<th></th>
<th>Multivariable analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>aHR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Cirrhosis status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recognized cirrhosis</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Unrecognized cirrhosis</td>
<td>1.35 (1.25 to 1.46)</td>
<td>&lt;0.001</td>
<td>1.17 (1.08 to 1.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No cirrhosis</td>
<td>1.11 (1.02 to 1.21)</td>
<td>0.02</td>
<td>0.84 (0.76 to 0.93)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Male sex (ref. female)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.02 to 1.03)</td>
<td>&lt;0.001</td>
<td>1.01 (1.00 to 1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Race/ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>1.01 (0.89 to 1.15)</td>
<td>0.86</td>
<td>0.97 (0.85 to 1.12)</td>
<td>0.70</td>
</tr>
<tr>
<td>Non-Hispanic API/Others</td>
<td>0.65 (0.59 to 0.72)</td>
<td>&lt;0.001</td>
<td>0.77 (0.69 to 0.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.94 (0.85 to 1.04)</td>
<td>0.22</td>
<td>0.92 (0.83 to 1.02)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Census Poverty Level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5%</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>5% to &lt;10%</td>
<td>1.14 (1.03 to 1.26)</td>
<td>0.01</td>
<td>1.06 (0.95 to 1.17)</td>
<td>0.30</td>
</tr>
<tr>
<td>10% to &lt;20%</td>
<td>1.12 (1.02 to 1.24)</td>
<td>0.02</td>
<td>1.03 (0.93 to 1.14)</td>
<td>0.55</td>
</tr>
<tr>
<td>20% to 100%</td>
<td>1.14 (1.03 to 1.26)</td>
<td>0.009</td>
<td>1.08 (0.97 to 1.20)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Rural-Urban</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metro &gt;1 million</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Metro 250k to 1 million</td>
<td>1.15 (1.06 to 1.25)</td>
<td>0.001</td>
<td>1.14 (1.05 to 1.24)</td>
<td>0.002</td>
</tr>
<tr>
<td>Metro &lt;250k</td>
<td>1.23 (1.09 to 1.39)</td>
<td>0.001</td>
<td>1.07 (0.94 to 1.21)</td>
<td>0.29</td>
</tr>
<tr>
<td>Non-Metro/Rural</td>
<td>1.28 (1.15 to 1.42)</td>
<td>&lt;0.001</td>
<td>1.12 (1.00 to 1.25)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>NCI comorbidity index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0 to 2)</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Moderate (&gt;2 to 4)</td>
<td>1.23 (1.11 to 1.35)</td>
<td>&lt;0.001</td>
<td>1.09 (0.99 to 1.20)</td>
<td>0.09</td>
</tr>
<tr>
<td>High (&gt;4)</td>
<td>1.60 (1.43 to 1.78)</td>
<td>&lt;0.001</td>
<td>1.35 (1.21 to 1.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Etiology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>NAFLD</td>
<td>1.37 (1.27 to 1.49)</td>
<td>&lt;0.001</td>
<td>1.19 (1.09 to 1.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALD</td>
<td>1.36 (1.24 to 1.50)</td>
<td>&lt;0.001</td>
<td>1.18 (1.06 to 1.30)</td>
<td>0.001</td>
</tr>
<tr>
<td>HBV</td>
<td>0.87 (0.73 to 1.03)</td>
<td>0.10</td>
<td>1.08 (0.91 to 1.28)</td>
<td>0.40</td>
</tr>
<tr>
<td>Other/None</td>
<td>1.21 (1.06 to 1.38)</td>
<td>0.004</td>
<td>1.13 (0.98 to 1.29)</td>
<td>0.09</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.09 (1.02 to 1.17)</td>
<td>0.01</td>
<td>1.04 (0.97 to 1.12)</td>
<td>0.29</td>
</tr>
<tr>
<td>Early stage*</td>
<td>0.46 (0.42 to 0.50)</td>
<td>&lt;0.001</td>
<td>0.54 (0.50 to 0.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Curative treatment†</td>
<td>0.27 (0.25 to 0.30)</td>
<td>&lt;0.001</td>
<td>0.30 (0.28 to 0.33)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aHR, adjusted hazard ratio; ALD, alcoholic liver disease; API, Asian/Pacific Islander; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; Metro, metropolitan; NAFLD, nonalcoholic fatty liver disease; NCI, National Cancer Institute. 
*Early-stage HCC was defined as a single tumor, less than or equal to 5 cm in diameter without vascular invasion or extrahepatic. †Curative treatments were defined as liver resection, liver transplantation, and tumor ablation.
Black patients may partly explain underlying racial ethnic disparity in HCC surveillance, curative treatment, and overall survival as reported in previous studies. Consistent with other studies, our previous study using SEER-Medicare Linked Database showed that non-Hispanic Black race is inversely associated with HCC surveillance receipt, early stage HCC detection, and curative treatment receipt. A comprehensive approach to monitoring and eliminating racial-ethnic disparities in early recognition of cirrhosis and surveillance implementation is urgently needed to reduce disparities in early HCC detection and prognosis. Other than demographic factors, we found that higher comorbidities and diabetes were associated with lower odds of having unrecognized cirrhosis. This could be explained by higher healthcare utilization in patients with comorbid conditions.

While under-recognition of cirrhosis is thought to be related to poor survival due to the lack of HCC surveillance, no study directly interrogates their association. The previous VA study showed HCC patients with unrecognized cirrhosis were 6.5 times more likely to be diagnosed with advanced stage HCC compared to with recognized cirrhosis, implying their unfavorable prognosis. Importantly, our study first confirmed the inferior survival of HCC patients with unrecognized cirrhosis, even after adjusting for tumor stage and treatment type. Therefore, earlier recognition of cirrhosis, particularly in the growing population of NAFLD patients will likely lead to improved overall survival after HCC diagnosis.

We acknowledge the inherent limitations of this retrospective study. First, compared to previous study by Walker et al., where a systematic electric chart review was performed, use of ICD-9 and ICD-10 codes in our current study to define cirrhosis and the underlying etiology of HCC could have led to misclassification. For example, some cirrhosis cases may have been recognized but could have been undercoded; codes for cirrhosis were not assigned within 6 months preceding HCC diagnosis may not guarantee the unrecognized cirrhosis. However, consistency of the findings between ours and Walker et al. confirmed the validity of the results. Secondly, Medicare population represents older individuals, and the study results might not be generalizable to younger patients with HCC. In addition, the stringent inclusion and exclusion criteria of this study might introduce selection bias. For instance, since most of the patients with HBV- or HCV-related HCC were diagnosed and deceased before 70 years old, patients with NAFLD-related HCC might have been overrepresented and burden of unrecognized cirrhosis could have been overestimated in the current study. Lastly, missing data on Child-Pugh score prevent us from performing granular subgroup analyses.

Despite these limitations, our study has the strength of including nationwide population compared to the previous study. We first confirmed the findings (i.e., NAFLD, Black race, and age are associated unrecognized cirrhosis) from the previous study in a larger cohort outside VA system. In addition, the majority of patients in the previous study had HCV-associated HCC, while few HCC patients with NAFLD were included. Our study included more balanced etiologies and 1,813 HCC patients had NAFLD, which could represent the trends of global rise of HCC patients attributed to NAFLD. Lastly, we enrolled the HCC patients diagnosed between 2011–2015, which follows the period (2005–2011) of the previous study, highlighting the issue of unrecognized cirrhosis remains unsolved and requires immediate intervention.

In summary, our results highlight that recognition of cirrhosis remains a common barrier to effective HCC surveillance implementation in the United States, particularly among patients with NAFLD-related HCC. We also validated the independent association between unrecognized cirrhosis and poor prognosis. Future studies and efforts are required to evaluate intervention strategies to better recognize cirrhosis at early stages to promote increased uptake of HCC surveillance programs and improve patients’ outcomes.

Authors’ contribution

Concept and design: Yang JD; Acquisition and Statistical analysis: Karim M; Drafting of the manuscript: Lee Y-T, Singal AG, Yang JD; Interpretation of data, Critical revision of the manuscript for important intellectual content: All authors; Administrative, technical, or material support: Yang JD; Supervision: Yang JD.

Acknowledgements

This study used the SEER-Medicare Linked Database. The authors acknowledge the efforts of the Centers for Disease Control and Prevention; National Program of Cancer Registries; National Cancer Institute; the Surveillance, Epidemiology, and End Results program; and the Medicare program in the creation of the SEER-Medicare Linked Database.
Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

**Conflicts of Interest**

Dr. Singal has been on advisory boards and served as a consultant for Wako Diagnostics, Glycotest, Exact Sciences, Roche, GRAIL, Genentech, Bayer, Eisai, BMS, Exelixis, AstraZeneca, and TARGET RWE. Dr Rich as served as consultant for AstraZeneca. Dr. Noureddin has been on the advisory board for 89BIO, Gilead, Intercept, Pfizer, Novo Nordisk, Blade, EchoSens, Fractyl, Terns, Siemens and Roche diagnostics; MN has received research support from Allergan, BMS, Gilead, Galmed, Galectin, Genfit, Conatus, Enanta, Madrigal, Novartis, Pfizer, Shire, Viking and Zydus; Dr. Noureddin is a minor shareholder or has stocks in Anaetos, Rivus Pharma and Viking. Dr. Yang provides consulting services for Exact Sciences, Gilead Sciences, Exelixis, and Eisai. Dr. Lee, Dr. Karim, Dr. Kum, and Dr. Park have no conflicts of interest to disclose.

**REFERENCES**


Taurocholic acid promotes hepatic stellate cell activation via S1PR2/p38 MAPK/YAP signaling under cholestatic conditions

Jing Yang1,*, Xujiao Tang1,*, Zhu Liang1, Mingzhu Chen1, and Lixin Sun2

1School of Life Sciences and Health Engineering, Jiangnan University, Wuxi, Jiangsu; 2Jiangsu Center Pharmacodynamic Research and Evaluation, China Pharmaceutical University, Nanjing, China

Study Highlights
- TCA activated quiescent HSCs into proliferative, migratory, contractile, and fibrogenic myofibroblasts.
- TCA promotes hepatic stellate cell activation via S1PR2/p38 MAPK/YAP signaling under cholestatic conditions.
- S1PR2 is the predominant S1PR expressed in HSCs and is upregulated under cholestatic conditions.
- Blockage of S1PR2 attenuated liver injury and fibrogenesis in DDC-induced liver fibrosis.
- S1PR2 is a potential therapeutic target in cholestatic liver disease.
INTRODUCTION

Cholestasis is mainly characterized by disturbances in bile salt synthesis, secretion and excretion, which lead to intrahepatic accumulation of bile salts and progressive liver damage or fibrosis.1 Cholestasis is a prominent manifestation of end-stage liver cirrhosis.2 The extension of fibrotic lesions to other bile ducts and the hepatic sinusoidal system around the por-

**Background/Aims:** Disrupted bile acid regulation and accumulation in the liver can contribute to progressive liver damage and fibrosis. However, the effects of bile acids on the activation of hepatic stellate cells (HSCs) remain unclear. This study investigated the effects of bile acids on HSC activation during liver fibrosis, and examined the underlying mechanisms.

**Methods:** The immortalized HSCs, LX-2 and JS-1 cells were used for the in vitro study. in vitro, the adeno-associated viruses adeno-associated virus-sh-S1PR2 and JTE-013 were used to pharmacologically inhibit the activity of S1PR2 in a murine model of fibrosis induced by a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet. Histological and biochemical analyses were performed to study the involvement of S1PR2 in the regulation of fibrogenic factors as well as the activation properties of HSCs.

**Results:** S1PR2 was the predominant S1PR expressed in HSCs and was upregulated during taurocholic acid (TCA) stimulation and in cholestatic liver fibrosis mice. TCA-induced HSC proliferation, migration and contraction and extracellular matrix protein secretion were inhibited by JTE-013 and a specific shRNA targeting S1PR2 in LX-2 and JS-1 cells. Meanwhile, treatment with JTE-013 or S1PR2 deficiency significantly attenuated liver histopathological injury, collagen accumulation, and the expression of fibrogenesis-associated genes in mice fed a DDC diet. Furthermore, TCA-mediated activation of HSCs through S1PR2 was closely related to the yes-associated protein (YAP) signaling pathway via p38 mitogen-activated protein kinase (p38 MAPK).

**Conclusions:** TCA-induced activation of the S1PR2/p38 MAPK/YAP signaling pathways plays a vital role in regulating HSC activation, which might be therapeutically relevant for targeting cholestatic liver fibrosis. (Clin Mol Hepatol 2023;29:465-481)

**Keywords:** Taurocholic acid; Sphingosine 1-phosphate receptor 2; Yes-associated protein; Hepatic stellate cells; Liver fibrosis

**Corresponding author:** Jing Yang
School of Life Sciences and Health Engineering, Jiangnan University, Wuxi, Jiangsu 214122, China
Tel: +86-0510-85329042, Fax: +86-0510-85329042, E-mail: yangjing@jiangnan.edu.cn
https://orcid.org/0000-0003-3068-6595

Lixin Sun
Jiangsu Center Pharmacodynamic Research and Evaluation, China Pharmaceutical University, Nanjing 210009, China
Tel: +86-025-83271057, Fax: +86-025-83271057, E-mail: slxcpu@126.com
https://orcid.org/0000-0002-3442-2602

*These two authors contributed equally to this paper.

**Editor:** Ji Won Han, The Catholic University of Korea College of Medicine, Korea
Received : Oct. 21, 2022 / Revised : Dec. 29, 2022 / Accepted : Feb. 16, 2023

**Abbreviations:**
HSCs, hepatic stellate cells; AAV, adeno-associated virus; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; TCA, taurocholic acid; YAP, yes-associated protein; p-YAP, phospho-YAP; p38 MAPK, p38 mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; FXR, farnesoid X receptor; LXR, liver X receptor; S1PR2, sphingosine 1-phosphate receptor 2; α-SMA, α-smooth muscle actin; FBS, fetal bovine serum; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ROCK, Rho kinase; HBV, hepatitis B virus; OCA, obeticholic acid; UDCA, ursodeoxycholic acid; NASH, nonalcoholic steatohepatitis; SHP, small heterodimer partner; TBA, total bile acids

https://doi.org/10.3350/cmh.2022.0327
http://www.e-cmh.org
tal area can hinder the secretion and excretion of bile and cause local hepatic tissue microcirculation disorders, further exacerbating cholestasis in a vicious cycle. Although tremendous efforts have been invested in the clinical management of these diseases and some promising agents are in development, the underlying pathogenesis and biomarkers of disease progression remain poorly understood.

Growing evidence indicates elevated bile acid levels in cholestasis are an independent profibrogenic factor. High bile acid concentrations in cholestatic liver disease cause liver parenchymal injury by inducing apoptosis and necrosis in hepatocytes. Unlike hepatocytes, hepatic stellate cells (HSCs), which are the major cellular source of matrix protein-secreting myofibroblasts, show increased proliferation in response to elevated bile acid concentrations and are resistant to bile acid-mediated cell death. However, few studies have explored the potential direct activation of HSCs by bile acids, and the molecular pathways mediating bile acid-induced effects in HSCs remain elusive. Svegliati-Baroni et al. found that bile acids promoted cell proliferation through epidermal growth factor receptor (EGFR) in HSCs, but the authors did not investigate the effects of bile acids on the myofibroblast-like functions of HSCs, such as cell migration, contraction and extracellular matrix protein secretion. How bile acid signals regulate the key events involved in HSC activation and liver fibrosis under cholestatic conditions is not well understood.

As important signaling molecules, bile acids usually regulate hepatic metabolism by activating nuclear receptors (farnesoid X receptor [FXR], liver X receptor [LXR]) or cell membrane receptors (GPBAR1 [TGR5], sphingosine 1-phosphate receptor 2 [S1PR2]). Given that HSCs lack bile acid transporters and thus cannot take up bile acids, they can only be taken up into the cell through the cell membrane receptors involved in HSC activation under cholestatic conditions. Recent studies have identified S1PR2 as a bile acid-activated receptor that plays a unique role in liver pathophysiology. S1PR2 activation is a critical component of cholangiocyte proliferation under cholestatic conditions. In the serum of murine obstructive cholestasis models and liver cirrhosis patients, taurocholic acid (TCA) is one of the most abundant bile acids. Previous studies using homology modeling identified TCA as an agonist predicted to hydrogen bond to Leu173 of S1PR2. However, whether bile acids such as TCA and S1PR2 are correlated in HSC transdifferentiation and activation remains unclear.

In the current study, we examined the effect of bile acids, especially TCA, on the activation of HSCs and the progression of fibrosis under cholestatic conditions. Our findings suggest that TCA/S1PR2-mediated signaling pathways play key roles in HSC activation and cholestatic liver fibrosis. We also described the principles of HSC activation under cholestatic conditions and identified prospects for novel diagnostics and therapies among patients with cholestatic liver fibrosis.

MATERIALS AND METHODS

Reagents and antibodies

Taurocholate acids and diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate (DDC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Recombinant human TGF-β, Y27632, U0126 and SB203580 were obtained from MCE MedChemExpress (Monmouth Junction, NJ, USA). JTE-013 and CAY1044 were purchased from Cayman Chemicals (Ann Arbor, MI, USA). The α-SMA, collagen 1, p-YAP (Ser127), and YAP antibodies were obtained from Cell Signaling Technology (Danfoss, MA, USA). β-actin, S1PR2 and Lamin B1 antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). HRP-linked anti-mouse and anti-rabbit IgG antibodies were obtained from Beyotime Biotechnology (Nanjing, China).

Cell lines and cell culture

The human HSC line LX-2 and the mouse HSC line JS-1 were obtained from FengHui Biological Co., Ltd. (Hunan, China). HepG2 cells were obtained from the China Cell Bank (Shanghai, China). All cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS and penicillin–streptomycin at 37°C in a 5% CO₂ incubator.

Cell viability assay

LX-2, JS-1 or HepG2 cells were cultured in 96-well plates in serum-free medium overnight and were treated with various concentrations of TCA (0, 12.5, 25, 50, 100, 200, 400, 800 µM) in the presence or absence of specific antagonists of individual S1PRs for 24 h. Cell Counting Kit-8 (Vazyme Biotech, Nanjing, China) assays were used to evaluate cell viability. The OD
values at 450 nm were measured using a multilabel plate counter (Perkin Elmer, Waltham, MA, USA).

**Wound healing assay**

Three parallel lines were drawn on the bottom of each well of a 24-well plate, and then cells were seeded in the 24-well plate and cultured overnight (to more than 80% confluence). A pipette was used to form wounds in each well. The cells were treated with TCA (100 µM) in the presence or absence of specific antagonists of individual S1PRs. During the experiment, typical kinetic updates were recorded at 4 hours intervals, and photographs of the wounds were obtained. Cell migration was determined by measuring the decrease in the width of the corresponding scratch using ImageJ software as described previously.16

**Collagen gel contraction assay**

Type I collagen from rat tails (5 mg/mL; Absin Bioscience Inc., Shanghai, China), PBS (10×) reconstitution buffer and the cell suspension were adjusted according to the manufacturer's protocol. The final cell concentration was 4x10^5 cells/mL. After gelation, medium containing 100 µM TCA was added to the gel in each well and incubated for 24 hours. The gels were photographed, and the collagen gel area was measured.

**Western blot analysis**

Total proteins were extracted from liver tissue and cells using RIPA lysis buffer (Beyotime Biotechnology), quantified by the BCA method (Beyotime Biotechnology), and analyzed by Western blot using specific primary and secondary antibodies, and the immunoreactive bands were visualized using an ECL chemiluminescent kit (Absin Bioscience Inc.) and ImageJ software.

**Quantitative real-time polymerase chain reaction**

Total RNA was extracted from liver tissue and cells by TRIzol reagent, reverse-transcribed using a HiScript II Q RT SuperMix for qPCR kit (Vazyme Biotech) and a ChamQ Universal SYBR qPCR Master Mix kit (Vazyme Biotech). β-actin was used as an internal control. The relative expression level of each target gene was calculated by the 2-ΔΔCT method. The primer sequences used are presented in Supplementary Table 1.

**Cellular YAP translocation assay**

The cells were plated on coverslips, cultured overnight and treated with TCA (100 µM) in the presence or absence of JTE-013 (10 µM) for 4 hours. Then, the cells were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, blocked with 5% BSA for 1 hour, and incubated with YAP antibodies (1:200 dilution) overnight at 4°C. After the coverslips were washed, Alexa Fluor 488 secondary antibodies were added and incubated for 1 hour at 37°C. Finally, the coverslips were sealed with an anti-fluorescence quencher containing DAPI, and the cells were observed and photographed with a Carl Zeiss LSM880 microscope.

**RNA interference**

The cells were cultured overnight to 50% confluence and transfected with a shRNA lentivirus vector targeting the S1PR2 or YAP gene. The primer sequences used are presented in Supplementary Table 2. After 48 hours, the cells were used for further experiments.

**Animals and experimental design**

All animal experiments were approved by the Animal Research Committee of Jiangnan University (JN.No2019 93010501210[252]) and were performed in accordance with the principles of care and the use of laboratory animals. ICR mice (male, 8 weeks) were purchased from Cavens Laboratory Animal Co. Ltd. (Changzhou, China). For the JTE-013 interference model, the mice were fed a control diet or a DDC-supplemented diet (0.1%) for 4 weeks to induce advanced biliary fibrosis. Then, 10 mg/kg B.W. JTE-013 was administered intraperitoneally one day before DDC diet administration and three times per week for 4 weeks (n=7 per group). For the S1PR2 knockdown mouse model, the mice were injected via the tail vein with adeno-associated virus (AAV8) carrying the S1PR2-targeting shRNA or scramble control shRNA (SHX211125A2; KeyGEN Biotech Company, Nanjing, China). After one week, cholestatic liver fibrosis was induced by DDC in accordance with our study.
Determination of hepatic enzyme levels

ALT levels, AST activity and total bile acids (TBA) in serum were analyzed using ALT, AST and TBA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

Determination of hepatic hydroxyproline levels

Hepatic hydroxyproline levels were quantified using a kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer’s protocol.

Liver histopathology

Liver tissues were fixed in 4% paraformaldehyde and sectioned at a thickness of 5 μm. The sections were stained with H&E to evaluate the degree of liver injury using light microscopy. For Masson’s trichrome staining, the sections were stained with a Masson’s trichrome stain kit (Absin Bioscience Inc.) according to the kit directions.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8 software, and all data are expressed as the mean±SEM. Comparisons among multiple groups were conducted using one-way analysis of variance followed by Bonferroni’s multiple comparison test. A P-value less than 0.05 was considered statistically significant.

RESULTS

Taurocholate acid promotes HSC activation in vitro

To further investigate the role of TCA in HSC transdifferentiation and activation, we selected two hepatic stellate cell lines: LX-2 and JS-1. As shown in Figure 1A, TCA dose-dependently stimulated HSC proliferation, as shown by CCK-8 assays. HSC proliferation became evident at low concentrations of TCA (12.5–200 μM) in LX-2 cells compared with that in the control and at high concentrations of TCA (>200 μM) in JS-1 cells (Fig. 1A). In contrast, TCA at the same concentration (>200 μM) both increased the number of HepG2 cells and induced considerable cell death (Fig. 1A). These results indicated that high bile acid concentrations under cholestatic conditions stimulated HSC proliferation and induced apoptosis in hepatocytes.

Next, we sought to determine whether TCA plays a role in HSC motility and contractility, which are two common features of myofibroblasts, by using wound healing assays and collagen gel contraction assays, respectively. As expected, TCA (100 μM) administration greatly increased the number of migrated LX-2 cells, especially after 36 hours (Fig. 1B). Similar to the findings in JS-1 cells, TCA markedly promoted cell migration at 24 hours compared to that in the control (Fig. 1C), indicating that TCA increased HSC mobility. Moreover, treatment with TCA clearly reduced the gel surface area compared to that in the control LX-2 cells, confirming that TCA enhanced collagen gel contractility (Fig. 1D).

Given that the fibrogenic functions of activated HSCs are critical initiators of hepatic fibrosis, western blot was used to assess the protein levels of α-smooth muscle actin (α-SMA) and collagen 1 after TCA administration in HSC. We found that TCA increased the protein expression of α-SMA and collagen 1 in LX-2 and JS-1 cells, and expression of these factors was upregulated in the positive control groups grown with serum (10% fetal bovine serum [FBS]) or treated with TGF-β (10 ng/mL) (Fig. 1E, F). Moreover, real-time PCR showed that the mRNA levels of α-SMA and collagen 1 were dramatically increased by TCA in LX-2 and JS-1 cells, respectively (Fig. 1E, F). Taken together, these results indicate that TCA activated quiescent HSCs into proliferative, migratory, contractile, and fibrogenic myofibroblasts.

S1PR2 is the predominant S1PR expressed in HSCs and is upregulated under cholestatic conditions

First, we examined the dynamic expression of bile acid-related receptors and found that the levels of the bile acid importer Sodium taurocholate cotransporting polypeptide (NTCP) and nuclear receptor FXR were extremely low in quiescent and activated HSCs (Supplementary Fig. 1), which inhibited bile acid uptake by HSCs. Therefore, bile acids can promote HSC activation only through cell membrane receptor signaling pathways. TGR5 and S1PR2 are the main G protein-coupled membrane receptors that are responsive to bile...
acids (Supplementary Fig. 2). TGR5 is expressed only in activated HSCs (Supplementary Fig. 2) and is activated by unconjugated bile acids.\textsuperscript{17,18} Our previous study showed that S1PR2 is the predominant S1PR in hepatocytes and cholangiocytes and that S1PR2 mediates TCA-induced cholangiocyte proliferation.\textsuperscript{13,15} In this study, PCR analysis and agarose gel electrophoresis was performed to assess the expression levels of S1PR2 and S1PR3.

**Figure 1.** TCA stimulates HSC activation. Cell viability was examined in response to different concentrations of TCA in LX-2, JS-1 or HepG2 cells (A). Cell motility was examined in response to TCA (100 μM) in LX-2 (B) and JS-1 (C) cells. Cell contractility was examined in response to TCA (100 μM) in LX-2 (D) cells. Protein and mRNA levels of α-SMA and collagen 1 in LX-2 (E) and JS-1 (F) cells after TCA treatment (100 μM). The data shown are the mean±SEM. TCA, taurocholic acid; HSCs, hepatic stellate cells; α-SMA, α-smooth muscle actin. *P<0.05, **P<0.01, ***P<0.005 compared with the control group; n=4.
phoresis showed that S1PR1, S1PR2, and S1PR3 were expressed at detectable levels, and that S1PR2 and S1PR3 were the most abundant S1PRs in LX-2 and JS-1 cells (Fig. 2A).

Given that HSC activation is a hallmark of hepatic fibrosis, we further correlated S1PR expression with HSC activation. Treatment with 10% FBS, TGF-β or TCA was used to activate HSCs in vitro. As demonstrated by real-time PCR, expression of only S1PR2 was significantly increased in activated LX-2 and JS-1 cells, especially after TCA treatment (Fig. 2B). Subsequently, we determined whether S1PR2 expression was altered in mice with cholestatic liver fibrosis by feeding them a 0.1% DDC-supplemented diet for 2 weeks or 4 weeks (Supplementary Fig. 3). Interestingly, the mRNA expression of S1PR2 was significantly upregulated in the liver and primary HSC in the DDC-stimulated groups, and the highest response occurred at 4 weeks (Fig. 2C). Finally, we performed immunofluorescence analysis of hepatic S1PR2 (red) and α-SMA (green) in 0.1% DDC-treated mice with cholestatic liver fibrosis. As shown in Figure 2D, S1PR2 protein expression was high in activated HSCs under cholestatic conditions.

**S1PR2 mediates HSC activation induced by TCA**

We next used specific S1PR antagonists to determine which S1PR subtypes are involved in TCA-induced HSC activation. As shown in Fig. 3A, pretreatment with the S1PR2 antagonist JTE-013 reversed TCA-induced cell proliferation, whereas the specific S1PR3 antagonist CAY10444 failed to induce this ef-

**Figure 2.** S1PR2 is the predominant S1PR expressed in HSCs and is upregulated under cholestatic conditions. S1PR expression in LX-2 and JS-1 cells was examined by PCR and agarose gel electrophoresis (A). Dynamic mRNA expression of S1PR2 and S1PR3 in quiescent and activated LX-2 and JS-1 cells (B). ICR mice were subjected to 0.1% DDC feeding for two weeks or four weeks. Total RNA was isolated. Mouse primary HSCs were isolated from sham control or DDC-fed mice (4 weeks) (C). The mice were fed a control diet or a DDC-supplemented diet (0.1%) for 2 or 4 weeks to induce advanced biliary fibrosis. S1PR2 mRNA levels were detected by real-time RT-PCR and normalized using GAPDH. Immunofluorescence analysis of hepatic α-SMA (green) and S1PR2 (red) in mice with DDC-induced liver fibrosis (D). The data shown are the mean±SEM. S1PR2, sphingosine 1-phosphate receptor 2; HSCs, hepatic stellate cells; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; α-SMA, α-smooth muscle actin; FBS, fetal bovine serum; TCA, taurocholic acid. *P<0.05, **P<0.01 compared with the control group; n=3.
Figure 3. S1PR2 mediates HSC activation induced by TCA. The viability of LX-2 or JS-1 cells that were preincubated with or without JTE-013 or CAY10444 and stimulated with TCA (12.5, 25, 50, 100 μM) (A). The motility of LX-2 or JS-1 cells that were preincubated with or without JTE-013 or CAY10444 and stimulated with TCA (100 μM) (B). The contractility of LX-2 cells (C) that were preincubated with or without JTE-013 or CAY10444 and stimulated with TCA (100 μM). The protein and mRNA levels of α-SMA and collagen 1 in LX-2 (D) and JS-1 (E) cells that were preincubated with or without JTE-013 and stimulated with TCA (100 μM). RT-PCR analysis of S1PR2, collagen 1 and α-SMA mRNA levels in LX-2 (F) and JS-1 cells (G) treated with lentivirus expressing S1PR2 shRNA or vector control after TCA treatment (100 μM). The data shown are the means±SEM. S1PR2, sphingosine 1-phosphate receptor 2; HSCs, hepatic stellate cells; TCA, taurocholic acid; α-SMA, α-smooth muscle actin; FBS, fetal bovine serum. *P<0.05, **P<0.01, ***P<0.005 compared with the control group. #P<0.05, ##P<0.01, ###P<0.005 compared with the TCA-only group; n=4.
fect in LX-2 or JS-1 cells. The administration of JTE-013 abrogated the migratory response of HSCs by TCA in LX-2 and JS-1 cells (Fig. 3B). In contrast, stimulation with CAY10444 had little effect on TCA-induced HSC migration, and the inhibitory effect of CAY10444 on the HSC migratory response induced by TCA was much lower than that of JTE-013 (Fig. 3B).

In LX-2 cells, pretreatment with JTE-013 or CAY10444 inhibited the potent increase in the contraction of HSC-embedded collagen gel in response to TCA (100 µM) and increased collagen gel surface area compared to that in the TCA-alone group, but the inhibitory effect of JTE-013 was greater than that of CAY10444, indicating that S1PR2 mediated the effect of TCA on HSC contraction (Fig. 3C).

Similar to the effects on extracellular matrix protein production, JTE-013 completely blocked TCA-induced protein expression of α-SMA and collagen 1 in LX-2 and JS-1 cells (Fig. 3D, E). RT-PCR showed that the TCA-induced increase in α-SMA and collagen 1 mRNA levels was inhibited by JTE-013-mediated downregulation of S1PR2 expression (Fig. 3D, E). S1PR2 shRNA also inhibited these TCA-induced fibrogenic effects of activated HSCs (Fig. 3F, G). RT-PCR confirmed the specific suppression of S1PR2 by this shRNA (Fig. 3F, G), and treatment with TCA failed to produce similar effects. Taken together, these data showed that TCA acts through S1PR2 to regulate HSC proliferation, migration, contraction, and production of extracellular matrix proteins.

**Figure 4.** Pharmacological inhibition of S1PR2 by JTE-013 alleviated the severity of liver injury in mice with DDC-induced liver fibrosis. A scheme of the mouse model is shown (A). Representative images of hepatic H&E staining and Masson’s trichrome staining are shown (B). Scale bars, 100 µm. Note the collagen fiber in the liver lobule (framed in green) preceding the development of portal-portal fibrous bridges (highlighted in yellow). Higher magnification view. Hepatic hydroxyproline levels (C). RT-PCR analysis of hepatic collagen 1, TIMP-1, TGF-β and CYP2B10 mRNA levels was carried out (D). Immunofluorescence staining showing hepatic α-SMA expression (E). The data shown are the mean±SEM. S1PR2, sphingosine 1-phosphate receptor 2; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; cv, central vein; α-SMA, α-smooth muscle actin. **P<0.01, ***P<0.005 compared with the control group. #P<0.05, ##P<0.01 compared with the DDC model group; n=5-7.
Pharmacological inhibition of S1PR2 by JTE-013 alleviated the severity of liver injury in mice with DDC-induced liver fibrosis

Given the effect of S1PR2 signaling on HSC activation and the role of HSCs in fibrotic responses, we next assessed the potential impact of S1PR2 antagonists on cholestatic liver fibrosis. We fed mice a 0.1% DDC diet in the presence or absence of JTE-013 for 4 weeks (Fig. 4A). Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are markers of liver injury, were decreased by the administration of JTE-013 in the DDC model group (Supplementary Fig. 4). H&E- and Masson’s trichrome-stained liver sections from all groups of mice indicated that treatment with JTE-013 significantly decreased bile duct proliferation, inflammatory cell infiltration and fibrotic regions in injured livers (Fig. 4B). The level of hydroxyproline in liver tissues was markedly attenuated in JTE-013-treated mice compared with DDC model mice ([122.9±7.18] μg/g, P<0.05) (Fig. 4C). The mRNA expression levels of fibrosis markers (collagen α1, TIMP-1, TGF-β and CYP2E10) were markedly elevated in the livers of mice fed a DDC-containing diet, and S1PR2 blockade after JTE-013 exposure decreased these responses (Fig. 4D). Finally, we performed immunofluorescence analysis of hepatic α-SMA (green), which confirmed that JTE-013 administration reversed the increase in the number of α-SMA-positive cells (activated HSCs) in mice with DDC-induced liver fibrosis (Fig. 4E, Supplementary Fig. 5). These results showed that pharmacologic inhibition of S1PR2 by JTE-013 attenuated liver injury and fibrogenesis in vivo.

S1PR2 knockdown alleviated liver injury in mice with cholestatic liver fibrosis

Considering the possible off-target effects of JTE-013, we next used gene delivery methods with recombinant adeno-associated virus (AAV) vectors to inhibit S1PR2 function (Fig. 5A). Wild-type mice injected with recombinant adenovirus carrying S1PR2 via the tail vein had significantly decreased S1PR2 expression compared to mice that received Ad-Vector (Fig. 5B). Serum levels of ALT and AST and the levels of proinflammatory cytokines, including TNF-α and IL-6, were also increased in the DDC model group but were decreased in AAV-shS1PR2 mice (Supplementary Fig. 6). Silencing S1PR2
dramatically alleviated bile duct proliferation and inflammatory cell infiltration in AAV-S1PR2 shRNA-DDC mice compared to DDC model mice (Fig. 5C). Masson’s trichrome staining showed a significant reduction in collagen deposition in the livers of AAV-S1PR2 shRNA-infected mice compared to those of single DDC model mice (Fig. 5C), which was consistent with decreased hepatic hydroxyproline levels (Fig. 5D). DDC mice treated with AAV-S1PR2 shRNA exhibited a significant decrease in hepatic mRNA expression of fibrosis markers, including collagen 1, TIMP-1, TGF-β, and CYP2B10, compared to that in DDC model mice (Fig. 5E). Finally, immunofluorescence staining for hepatic α-SMA (green) confirmed that the number of α-SMA-positive cells (activated HSCs) in DDC mice treated with AAV-S1PR2 shRNA was less than that in DDC mice treated with Ad-Vector (Fig. 5F). These results suggest that S1PR2 expression is essential for the development of liver fibrogenesis.

YAP mediates TCA/S1PR2 signaling during HSC activation and cholestatic liver fibrosis

Based on the report that the transcriptional coactivator yes-associated protein (YAP) is a powerful regulator of activated HSCs and to further investigate the role of YAP in the TCA-mediated S1PR2-related signaling pathway, LX-2 and JS-1 cells were treated with TCA for 4 h with or without JTE-013 stimulation. Figure 6A show that TCA decreased phospho-YAP (p-YAP) levels in the cytosol and increased nuclear YAP levels, strongly demonstrating TCA-induced YAP transactivation in LX-2 and JS-1 cells. As expected, JTE-013 significantly decreased TCA-induced YAP nuclear localization in LX-2 and JS-1 cells (Fig. 6A). Moreover, as demonstrated by immunofluorescence analysis, YAP was uniformly distributed throughout the entire cell in the control group, whereas after treatment with 100 μM TCA for 4 hours, YAP translocated to the nucleus in most HSCs (Fig. 6B, Supplementary Figs. 7, 8). However, when S1PR2 was blocked by JTE-013, YAP remained in the cytoplasm of HSCs (Fig. 6B, Supplementary Figs. 7, 8). Similarly, YAP target genes (e.g., CTGF and CyclinD1) were strongly expressed in HSCs that were treated with TCA and were weakly expressed in cells that were pretreated with JTE-013 or S1PR2 shRNA (Fig. 6C, D). Similarly, in DDC-supplemented mice, significantly fewer AAV-S1PR2 shRNA-infected mice showed nuclear YAP compared with the vehicle control group (Fig. 6E). This result, together with the data that AAV-S1PR2 shRNA infection led to lower CyclinD1 and CTGF levels, further shows that TCA-mediated S1PR2 activation and triggered YAP nuclear translocation and downstream effects (Fig. 6F). Finally, downregulation of YAP using a lentiviral shRNA specific to YAP completely blocked TCA-induced upregulation of CTGF and collagen 1 in JS-1 cells (Fig. 6G).

As a G protein-coupled receptor, S1PR2 couples with several different G-alpha subunits, such as Gα_{i/o}, Gα_{q} and Gα_{12/13}, and activates Rho kinase (ROCK), Erk or p38 MAPK. Our results showed that the selective p38 MAPK inhibitor SB203580 inhibited the TCA-induced increase in nuclear YAP protein levels in JS-1 and LX-2 cells (Fig. 7A). Moreover, Y27632 (a ROCK inhibitor) and U0126 (an ERK inhibitor) did not significantly inhibit the nuclear translocation of YAP. These results were consistent with the effect on YAP-target genes (e.g., CTGF and CyclinD1) and collagen 1 in JS-1 cells (Fig. 7B).

DISCUSSION

HSCs are resident perisinusoidal cells with slow proliferative rates that are distributed in the subendothelial space of Disse throughout the liver. Once liver injury occurs, signals sent from surrounding parenchymal cells, such as elevated bile acid concentrations, activate HSCs and promote HSC differentiation into proliferative, contractile, and fibrogenic myofibroblasts to repair the injured liver. A recent study demonstrated that HSCs were the main sources of fibroblasts (82–89%) in cholestatic-induced liver fibrosis models. However, it is unclear which signals are involved in HSC transdifferentiation under cholestatic conditions.

In this study, we demonstrated that the accumulation of bile acids, as occurs in obstructive jaundice, correlates with HSC transdifferentiation and the progression of fibrosis associated with cholestatic liver disease. We identified that bile acids, especially TCA, are profibrogenic factors that play essential roles in regulating the myofibroblast-like function of HSCs during cholestatic liver repair. Svegliati-Baroni et al. found that the effect of bile acids on HSC proliferation was closely related to their concentration. Under normal, noncholestatic conditions, bile acid concentrations such as 5 μM do not induce HSC proliferation; however, bile acids can promote HSC proliferation at concentrations that are commonly found in chronic cholestasis (approximately 200 μM). Consistent with these results, in the present study, the concentra-
Figure 6. YAP mediates TCA/S1PR2 signaling during HSC activation and cholestatic liver fibrosis. Western blot showing p-YAP and YAP levels in HSC (A). The YAP (green) distribution in the cells was observed by immunofluorescence staining (B). The nuclei of the cells were labeled with DAPI (blue). The mRNA levels of CTGF and CyclinD1 in LX-2 cells that were preincubated with or without JTE-013 and stimulated with TCA (100 μM) (C). RT-PCR analysis of CTGF and CyclinD1 mRNA levels in LX-2 cells treated with lentivirus expressing S1PR2 shRNA or vector control after TCA treatment (100 μM) (D). ICR mice were injected via the tail vein with adeno-associated virus carrying S1PR2-targeting shRNA or scramble control shRNA and were subjected to 4 weeks of DDC feeding as described in the Methods. Western blot showing p-YAP and YAP levels in the mice (E). RT-PCR analysis of hepatic cyclin D1, CTGF mRNA levels (F). JS-1 cells were treated with lentivirus expressing YAP shRNA or vector control after TCA treatment (100 μM). The mRNA levels of YAP, CTGF and collagen 1 were detected by real-time RT-PCR (G) and normalized to GAPDH, which acted as an internal control. The data shown are the mean±SEM. YAP, yes-associated protein; p-YAP, phospho-YAP; TCA, taurocholic acid; S1PR2, sphingosine 1-phosphate receptor 2; HSCs, hepatic stellate cells. *P<0.05 compared with the control group. **P<0.05 compared with the TCA-alone group; n=4.
tion of TCA (>200 μM) that dose-dependently stimulated HSC proliferation did not increase cell number or induce death in HepG2 cells (Fig. 1A). In addition, under pathological conditions (100 μM), TCA markedly promoted HSC transdifferentiation into proliferative, migratory, and contractile myofibroblasts, thus inducing profibrogenic transcriptional and secretory properties in human LX-2 and mouse JS-1 cells (Fig. 1). These results suggested that HSCs were resistant to the bile acid–induced signaling pathway, which could be attributed to a lack of the critical bile acid importer, suggesting that bile acid was not internalized. Sommerfeld et al. showed that cholestatic bile acids led to EGFR phosphorylation, triggered rapid NADPH oxidase activation and induced HSC proliferation in quiescent HSCs. In this study, S1PR2, a transmembrane G protein-coupled receptor, was required for TCA-induced proliferation, migration, contraction, and production of extracellular matrix proteins in LX-2 and JS-1 cells. Expression of S1PR2, the most abundant S1PR expressed in HSC, was significantly upregulated after HSC activation, especially after TCA treatment (Fig. 2). Previous studies have identified an affinity between TCA and S1PR2. JTE-013, a specific antagonist of S1PR2, and a shRNA specific to S1PR2 blocked TCA-induced HSC activation (Fig. 3). In addition, both S1PR2 deficiency induced by the injection of recombinant adenovirus carrying S1PR2 and antagonism of S1PR2 by JTE-013 selectively reduced hepatic fibrogenesis in mice with DDC-induced liver fibrosis (Figs. 4, 5). Kageyama et al. reported that an S1PR2 antagonist reduced portal vein pressure without affecting mean arterial pressure in rats with cirrhosis caused by bile duct ligation.

An earlier article published in Gut first described the presence of sodium-dependent taurocholate cotransporting polypeptide (Slc10a1/NTCP) on human liver HSCs obtained from liver fibrosis patients, and a positive correlation between NTCP expression levels and the severity of hepatic fibrosis was reported. Another study investigated how HSC NTCP mediated the uptake of bile acids and activated HSC function. Kunz et al. then used a specific and well-characterized NTCP inhibitory peptide (Myrcludex B) to perform bile salt uptake assays in LX-2 and U2OS cells overexpressing human NTCP. The results showed that unlike that of U2OS cells overexpressing human NTCP, the uptake of 3H-TCA in LX2 cells occurred at background levels and could not be blocked with Myrcludex B. In addition, when the cells were incubated with FITC-labeled Myrcludex B, TGF-β-activated LX2 cells did not show plasma membrane labeling, in contrast to NTCP-positive U2OS cells. These results suggest that no NTCP-positive cells were present in this cell population. Therefore, we detected the levels of NTCP and other bile acid-related receptors in quiescent and activated HSCs, which were extremely low (Supplementary Fig. 1). We agree with Kunz et al. that the experiments performed by Salhab et al. do not show a direct role of NTCP activity in stellate cells that contributes to fibrosis, and that further evidence is still needed. Interestingly, NTCP not only extracts the majority of conjugated bile acids but also mediates the entry of hepatitis B virus (HBV) into the liver. Myrcludex B and its ability to interfere with viral entry into hepatocytes are currently being tested in HBV/HDV clinical trials.

Obeticholic acid (OCA), the most potent FXR agonist, has been approved by the FDA and EMEA for the treatment of ursodeoxycholic acid (UDCA)-resistant patients with PBC. More recently, the results from a planned interim analysis of an ongoing phase 3 study of OCA for nonalcoholic steatohepatitis (NASH) showed that 25 mg of OCA significantly improved liver fibrosis (≥1 stage), compared to that in 12% of placebo patients. Although the phase III clinical trial of OCA for liver fibrosis with NASH reached one of its primary clinical endpoints, the response rate was not optimal. OCA was suggested to exert beneficial effects by activating FXR in hepatocytes and intestinal epithelial cells. More importantly, hepatocytes are highly expressed with more abundant FXR expression than seen in HSCs. Hepatocytes may regulate the activation of HSCs via the secretion of bile acids and apoptotic bodies, which suggests that FXR ligands also impede HSC activation indirectly by targeting FXR in hepatocytes. Moreover, OCA cannot directly protect hepatocytes from death receptor-engaged apoptosis, which is a core pathological event involved in stimulating fibrotic development. FXR protein levels are also reduced with the progression of fibrotic development and inflammation. In addition to FXR signaling, OCA directly inhibits NLRP3 inflammasome activation in macrophages, further suppressing inflammasome activation-elicited hepatic lipid accumulation and contributing to the amelioration of NASH. Some studies have shown that FXR is expressed in HSCs, where it functions as a transcription factor that regulates expression of the small heterodimer partner (SHP) gene and microRNA-29a, thereby reducing the expression of profibrotic genes, including Acta2 (encoding αSMA), transforming growth factor β1 (Tgfb1), collagen 1α1.
(Col1a1), Col1a2, tissue inhibitor of metalloprotease 1 (Timp1), and Timp2. However, these results were contradicted by later studies showing that culture-activated HSC were not responsive to FXR agonists; the expression of both SHP and ACTA2 in culture-activated HSCs remained unaffected after 24 h of in vitro stimulation with 1 or 100 μM OCA. In addition, a study from Fickert et al. showed undetectable FXR protein expression in HSCs and myofibroblasts in five mouse models of liver fibrosis of different etiologies and unchanged SHP mRNA expression in FXR ligand-treated myofibroblasts. Our experimental results are in accordance with previous studies; we also found that mRNA expression levels of FXR were very low, but did not remain unaffected after 24 hours of in vitro stimulation with 100 μM TCA (Supplementary Fig. 1). In 2020, Zhou et al. reported that enhanced SUMOylation of FXR in the process of HSC activation and fibrogenesis strongly compromises FXR signaling, providing insight into why OCA alone has limited effects against liver fibrosis.

As a central component of Hippo signaling, YAP is an important regulator of development and homeostasis in multiple tissues. YAP is relatively inactive in the healthy liver but is substantially activated in HSCs in response to liver injury. In vitro, YAP siRNA or the YAP inhibitor verteporfin block the differentiation of HSCs into myofibroblasts; in vivo, verteporfin slows liver fibrosis in mice. Mechanical signals such as ECM stiffness stimulate the Rho/ROCK pathway in the cytoplasm, promote YAP translocation, and activate downstream signaling pathways to induce cell differentiation into myofibroblasts. In addition, YAP activation stimulates glutaminolysis to direct quiescent HSC transdifferentiation and myofibroblastic HSC proliferation. These findings further confirm that YAP activation regulates HSC activation. In the signaling pathway upstream of YAP, several factors, including ROCK and S1PR2/p38 MAPK/YAP signaling axis in HSCs is essential for cholestatic liver fibrosis (C). The data shown are the mean±SEM. YAP, yes-associated protein; p-YAP, phospho-YAP; HSCs, hepatic stellate cells; TCA, taurocholic acid; S1PR2, sphingosine 1-phosphate receptor 2; ns, OOO. **P<0.01 compared with the control group. ##P<0.01 compared with the TCA-alone group; n=4.

Figure 7. p38 mitogen-activated protein kinase pathways mediate YAP nuclear translocation and HSC activation by TCA. Western blot showing p-YAP and YAP levels in JS-1 and LX-2 cells that were preincubated with or without Y27632 (ROCK inhibitor), U0126 (an ERK inhibitor) or SB203580 (a p38 inhibitor) and stimulated with TCA (100 μM) (A). mRNA levels of CTGF, CyclinD1 and collagen 1 in JS-1 cells that were preincubated with or without SB203580 and stimulated with TCA (100 μM) (B). Schematic diagram of the mechanism by which TCA activation of the S1PR2/p38 MAPK/YAP signaling axis in HSCs is essential for cholestatic liver fibrosis (C).
G-protein-coupled receptor signaling, mechanical signaling, and adhesive junctions, are well known to impact the activity of YAP. 47,48 In this study, we demonstrated that YAP-mediated HSC activation under cholestatic conditions occurred through S1PR2. Given that p38 MAPK was activated downstream of multiple S1PR2 signaling pathways and SB203580 (a p38 MAPK inhibitor) abrogated the TCA-induced increase in nuclear YAP protein levels, we concluded that the S1PR2/p38 MAPK/YAP signaling pathways regulated TCA-induced HSC activation (Fig. 7).

In summary, we found that conjugated bile acids stimulate the progression of liver fibrosis via S1PR2. These findings further highlight a promising correlation between accumulation of bile acids and progressive liver fibrosis, suggesting S1PR2 inhibition as a potential therapeutic target in cholestatic liver disease. Further studies are needed to confirm these results and explore their clinical implications.

Authors’ contribution

JY and LX conceived the study and designed the project. XT, ZL, and MC carried out the experiments and interpreted the data. XT wrote the paper. JY and LX revised the paper. All authors approved the final version of the manuscript.

Acknowledgements

This work was supported by Grants from the National Natural Science Foundation of China (No. 81900560, 81873084).

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


Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune-tolerant phase

Jeong-Ju Yoo¹*, Soo Young Park², Ji Eun Moon³, Yu Rim Lee⁴, Han Ah Lee⁵, Jieun Lee⁶, Young Seok Kim¹, Yeon Seok Seo⁶, and Sang Gyune Kim¹

¹Division of Gastroenterology and Hepatology, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon; ²Department of Internal Medicine, Kyungpook National University Hospital, Kyungpook National University School of Medicine, Daegu; ³Department of Biostatistics, Clinical Trial Center, Soonchunhyang University Bucheon Hospital, Bucheon; ⁴Department of Internal Medicine, College of Medicine, Ewha Womans University, Seoul; ⁵College of Medicine, Soonchunhyang University, Cheonan; ⁶Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea

Study Highlights

• Previous studies have attempted to define IT phase of chronic hepatitis B based on serum markers, only to attain inconsistent results, due to definitions of IT phase varying by studies. Therefore, it is essential to determine the definition of IT phase and criteria required for urgent treatment. Eighty-two (31.7%) out of 259 clinically suspected IT phase patients belonged to histologic IT phase. Among patients in IT phase identified by the AASLD and EASL criteria, 31.7% and 34.0% were in IT phase histologically, respectively. Old age, high AST and low albumin were useful for ruling out histologic IT phase. In conclusion, numerous patients in clinically suspected IT phase were not in IT phase histologically. Liver biopsy should be recommended to determine treatment for such patients.

Graphical Abstract

Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune-tolerant (IT) phase

- % of patients not belonging to histologic IT phase
  - AASLD criteria: 68.3%
  - EASL criteria: 66%
  - Risk factors not belonging to histologic IT phase
    - Age: High AST, Low albumin: 68.3%
    - Histologic IT phase: 31.7%

- Probability of histologic IT phase
  - Log-rank P<0.001

- HBV DNA level (IU/mL)
  - Age: <35 (n=64)
  - Age: ≥35 (n=195)
  - 10⁴-10⁶ IU/mL: 0% (n=11), 37.9%
  - ≥10⁷ IU/mL: 0% (n=15), 29.5%

Conclusion:

Age over 35 years, high AST, low albumin, low DNA level were associated with poor long-term outcomes. Additional histologic assessment needs to be considered.
INTRODUCTION

Advances in the knowledge of the evolution and phases of hepatitis B virus (HBV) acquired over the past 20 years, have allowed the gradual development of effective treatment options. As a result, the incidence of liver cirrhosis and liver cancer caused by hepatitis B has steadily decreased. However, some aspects of HBV are not clearly understood, such as the so-called immune-tolerant (IT) phase, characterized by high HBV deoxyribonucleic acid (DNA) levels and yet, persistently normal alanine aminotransferase (ALT). The IT phase is associated with a good prognosis in general and

Background/Aims: The histologic status of the immune-tolerant (IT) phase of chronic hepatitis B relative to long-term outcomes is unclear. This study aimed to discover how the serological criteria currently in use correspond to histologic criteria in determining the IT phase and indication for liver biopsy.

Methods: Patients in the serological IT phase determined by positive hepatitis B e antigen, hepatitis B virus (HBV) DNA ≥10^6 IU/mL, and normal or minimally elevated alanine aminotransferase (ALT) ≤60 IU/L, who underwent liver biopsy at three different hospitals were included. The distribution of the histologic IT phase, defined as fibrosis of stage 1 or less and inflammation of grade 1 or less, was compared with that of the serological IT phase. The risk factors for the incidence of liver-related events, such as hepatocellular carcinoma, liver cirrhosis, liver transplantation, and death, were also analyzed.

Results: Eighty-two (31.7%) out of 259 clinically suspected IT phase patients belonged to the histologic IT phase. Age over 35, high AST, and low albumin were useful for ruling out the histologic IT phase. Risk factors predicting liver-related events were age and significant fibrosis stage. There was no significant difference in the proportion of histologic IT phase and clinical prognosis between normal ALT and mildly elevated ALT groups. However, even in patients with normal ALT, age was an important factor in predicting the presence of the histologic IT phase.

Conclusions: A significant number of patients who belonged to the serological IT phase were not in the histologic IT phase. Patients over 35 years and those with high AST, low albumin, and low HBV DNA levels were more likely to experience poor long-term clinical outcomes. Therefore, additional histologic assessment should be considered. (Clin Mol Hepatol 2023;29:482-495)

Keywords: Fibrosis; Biopsy; Hepatitis B virus

Corresponding author: Sang Gyune Kim
Department of Gastroenterology and Hepatology, Digestive Research Center and Liver Clinic, Soonchunhyang University Bucheon Hospital, 170 Jomaru-ro, Wonmi-gu, Bucheon 14584, Korea
Tel: +82-32-621-5215, Fax: +82-32-621-6079, E-mail: mcnulty@schmc.ac.kr
https://orcid.org/0000-0001-8694-777X

Yeon Seok Seo
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Korea University College of Medicine, 73 Goryeodae-ro, Seongbuk-gu, Seoul 02841, Korea
Tel: +82-2-920-6608, Fax: +82-2-953-1943, E-mail: drseo@korea.ac.kr
https://orcid.org/0000-0003-4171-6331

*JJ Yoo and SY Park contributed equally as co-first authors.

Editor: Young-Suk Lim, University of Ulsan College of Medicine, Korea
Received: Oct. 19, 2022 / Revised: Nov. 30, 2022 / Accepted: Jan. 3, 2023

Abbreviations:
AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; EASL, European Association for the Study of the Liver; HBV, hepatitis B virus; HCC, Hepatocellular carcinoma; HR, hazard ratio; IRB, Institutional Review Board of our hospital; IT, immune-tolerant; OR, odds ratio

thus, not recommended for antiviral treatment in most
guidelines.\textsuperscript{2,5} The limited understanding of the IT phase is
evident in its arbitrary definition, which varies in different
guidelines. For example, in the European Association for the
Study of the Liver (EASL) guideline, the IT phase is defined as
high levels of HBV DNA of more than \(10^7\) IU/mL\textsuperscript{1}, whereas in the
American Association for the Study of Liver Diseases
(AASLD) guideline, the definition is HBV DNA of more than
\(10^6\) IU/mL.\textsuperscript{2} Numerous studies have suggested that IT pa-
tients defined by serological criteria are no longer immuno-
logically and histologically healthy, as supported by antigen-
specific T-cell deletion, inadequate clonal expansion of
effectort cells, and consequently, functional tolerance evi-
dent as immune tolerance. However, HBV-specific T-cell
responses, associated with clonal expansion of hepatocytes,
can sometimes be detected in the early stages of HBV infec-
tion, especially in increased random integration of HBV DNA
into infected hepatocytes. In addition, dysfunctional specific
T-cells found in both IT and immune active phases suggest
the ambiguity of boundaries that distinguish individual clin-
ical phases.

Most classifications of the IT phase in recent guidelines are
based on serum markers, especially HBV DNA and ALT and/or
aspartate aminotransferase (AST). While such laboratory-
ated criteria are inevitable, as liver biopsies cannot be per-
formed in all patients, it is well known that normal ALT and
high DNA cannot conclusively rule out indolent fibrosis.\textsuperscript{5}
Therefore, clinicians have debated continuously whether
treatment is necessary for patients in the IT phase.

Though a recent study published by Kim et al.,\textsuperscript{7} reported a
notable finding of poorer prognosis in the IT phase than in
the immune active phase with treatment, such findings may
be limited because the subjects’ qualification as IT phase pa-
tients is in question.\textsuperscript{8} Likewise, previous studies have at-
ttempted to define the IT phase based on serum markers,
only to attain inconsistent results because definitions of the
IT phase can vary by study. Therefore, it is essential to de-
termine the definition of the IT phase and the criteria required
for urgent treatment.

This study aimed to discover how well the serological crite-
rion in current use correspond to the histologic criteria in de-
termining the IT phase. In addition, we would like to suggest
potential indications that require liver biopsy among patients
in the serological IT phase.

\section*{Materials and Methods}

\subsection*{Patients and study protocol}

We collected consecutively the data of 312 chronic hepato-
tis B patients in the clinical IT phase from January 1994 to De-
ember 2017. The patients underwent a liver biopsy to deter-
mine the progression and treatment of hepatitis. Patients
who fulfilled the following inclusion criteria were eligible for
this study: (a) patients over the age of 20, who underwent liv-
er biopsy for chronic hepatitis B, (b) positive hepatitis B e an-
tigen (HBeAg) and high HBV DNA in at least two tests taken
more than six months apart, (c) normal or minimally elevated
ALT (<60 U/L) in at least two tests taken more than three
months apart, and (d) adequate histology in percutaneous
liver biopsy. Patients were excluded for the following condi-
tions: (a) HBV DNA lower than \(10^6\) IU/mL \((n=26)\), (b) high AST
or ALT (>60 IU/L) \((n=18)\), (c) inadequate histology \((n=2)\), (d)
co-infection with chronic hepatitis C or hepatitis D \((n=2)\) (e)
features of chronic liver disease or liver cirrhosis in imaging
studies \((n=4)\), and (f) prior or current evidence of hepatocel-
lar carcinoma (HCC) \((n=1)\). We included 259 patients that
met all criteria in the final selection (Supplementary Fig. 1).
Clinical, histologic, and laboratory records of the involved pa-
tients were reviewed retrospectively.

The study protocol was approved by the Institutional Re-
view Board of our hospital \((IRB number SCHBC-2020-
03-031-001, registration date: 7 April 2020). The study proto-
col conformed to the ethical guidelines of the World Medical
Association Declaration of Helsinki.

\subsection*{Liver biopsy and histology}

A liver biopsy was conducted when each investigator
deemed it necessary to evaluate the status or severity of
chronic hepatitis B, to determine the need for antiviral ther-
apy, or to identify autoimmune diseases or metabolic diseases.
Ultrasound-guided liver biopsy was performed by expert
hepatologists experienced with over 500 ultrasound proce-
dures and 100 liver biopsies. An adequate liver biopsy sample
was characterized by a length of 2 to 3 cm or more and the
inclusion of ten or more portal tracts.\textsuperscript{9,10} Specimens were
fixed in formalin and embedded in paraffin. The resulting
sections were stained with hematoxylin-eosin and Masson’s
trichrome. Each biopsy specimen was analyzed by patholo-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Histologic features of chronic hepatitis B in the immune active (IA) and immune tolerant (IT) phases.}
\end{figure}
gists from each institution with over ten years of experience. The inability to confirm the degree of agreement among pathologists is one of the major limitations of this retrospective study. Histologic grading and staging of the liver biopsy were described according to the standardized guideline proposed by the Korean Study Group for the Pathology of Digestive Disease.\textsuperscript{11} Fibrosis was assessed on a scale of 0 to 4: F0, no fibrosis; F1, portal fibrosis without septa; F2, periportal fibrosis; F3, septal fibrosis; and F4, liver cirrhosis. Inflammation was graded as none (G0), minimal (G1), mild (G2), moderate (G3), or severe (G4). Specimens of at least 20 mm in length and with 11 or more portal tracts included were considered eligible for interpretation in this study.\textsuperscript{12}

Outcomes, definition, and follow-up

The primary goal of the study was to evaluate the compatibility between pre-existing serological criteria and histologic criteria in determining the IT phase. The secondary goal was to find long-term prognostic factors in IT phase patients in association with outcomes of interest, such as liver cirrhosis, HCC, liver transplantation (LT), or death.

The definition of the IT phase according to the AASLD is as follows: (a) positive hepatitis B surface antigen for more than six months, (b) positive hepatitis B e antigen, (c) HBV DNA level higher than one million IU/mL and (d) normal (35 U/L for males and 25 U/L for females) or minimally elevated ALT.\textsuperscript{2}

The definition of the IT phase according to the EASL is as follows: (a) positive hepatitis B surface antigen for more than six months, (b) positive hepatitis B e antigen, (c) HBV DNA level higher than 10\textsuperscript{5} IU/mL, and (d) normal ALT (40 U/L).\textsuperscript{3}

The definition of the histologic IT phase is as follows: fibrosis of stage 1 or less and inflammation of grade 1 or less.

Liver cirrhosis was diagnosed by the presence of diffuse nodular surface or regeneration, dense fibrous septa, and architectural or hepatic vascular distortion in follow-up imaging studies, such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI).\textsuperscript{13} HCC was confirmed by the presence of typical features (arterial enhancement and portal-delayed washout in nodules of more than one centimeter) in imaging studies, including CT or MRI, or in histologic studies.\textsuperscript{14}

The index date was defined as the date of liver biopsy. The follow-up period was calculated from the index date to the date of the outcome of interest or the last follow-up date. Patients regularly attended the laboratory and/or abdominal ultrasound check-ups every three to six months. A liver-related event was defined as the occurrence of liver cirrhosis, HCC, LT, or death.

Statistical analysis

Frequencies and percentages were used for descriptive statistics. Statistical differences between groups were investigated using the χ\textsuperscript{2} test and Student’s t-test. Spearman’s analysis was used to investigate correlations between variables. The cumulative incidence of liver-related events between patients in and those not in the IT phase was estimated using the Kaplan-Meier method, and differences between the curves were compared using the log-rank test. Multivariable logistic regression analysis was used for risk factors to exclude patients in the histologic IT phase. Factors known to be effective in predicting the IT phase in previous studies (e.g., sex, age, body mass index, HBV DNA, platelet, AST, ALT, albumin, and total bilirubin) were analyzed. Cox proportional hazards model was used as the main analysis tool to calculate the incidence of liver-related events. Factors known to be associated with long-term liver-related events in previous studies (e.g., sex, age, HBV DNA, platelet, AST, ALT, albumin, total bilirubin, antiviral treatment, fibrosis stage, and inflammation grade) were analyzed. Multivariate models were created using variables that were clinically relevant and significant (P<0.01) in univariate analysis. All statistical analyses were performed using R (version 3.3.3, The R Foundation for Statistical Computing, Vienna, Austria) and SPSS software (ver. 21.0; SPSS Inc, Armonk, NY, USA). Statistical significance was defined as P<0.05.

RESULTS

Baseline characteristics

The baseline demographics and clinical characteristics of patients are summarized in Table 1. A total of 259 patients were analyzed, including 177 (68.3%) males. The patients were 42.7±12.5 years old on average. The median HBV DNA level was 2.4×10\textsuperscript{7} IU/mL (interquartile range [IQR] 2.5×10\textsuperscript{7}–8.5×10\textsuperscript{7}), and AST and ALT levels were 42 U/L (IQR 32–54) and 42 U/L (IQR 32–56), respectively. The median follow-up dura-
tion was 109 months (IQR 56–145).

The distribution of fibrosis stages and inflammation grades in patients is also presented in Table 1. Although all patients were not expected to exhibit advanced liver disease on imaging, advanced fibrosis (≥F3) was observed in as many as 101 (38.9%) patients. Similarly, given the low level of ALT, there was a significant number of patients (94, 36.3%) with inflammation more severe than the moderate grade.

### Table 1. Baseline characteristics of patients at enrollment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clinical IT phase (n=259)</th>
<th>Histologic IT phase (+) (n=82)</th>
<th>Histologic IT phase (-) (n=177)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>42.6±12.4</td>
<td>36.3±12.0</td>
<td>45.5±11.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.114</td>
</tr>
<tr>
<td>Male</td>
<td>177 (68.3)</td>
<td>62 (75.6)</td>
<td>115 (65.0)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>82 (31.7)</td>
<td>20 (24.4)</td>
<td>62 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Follow-up duration (mo)</td>
<td>109 (56–145)</td>
<td>120 (40–164)</td>
<td>106 (38–166)</td>
<td>0.887</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B e antigen positivity</td>
<td>259 (100)</td>
<td>82 (100)</td>
<td>177 (100)</td>
<td></td>
</tr>
<tr>
<td>HBV DNA (IU/mL)</td>
<td>2.4×10^6 (2.5×10^6–8.5×10^6)</td>
<td>5.5×10^6 (6.4×10^6–9.1×10^6)</td>
<td>1.6×10^6 (1.7×10^6–8.5×10^6)</td>
<td>0.012</td>
</tr>
<tr>
<td>10^6–10^7</td>
<td>33 (12.7)</td>
<td>10 (12.2)</td>
<td>23 (13.0)</td>
<td></td>
</tr>
<tr>
<td>10^7–10^8</td>
<td>68 (26.3)</td>
<td>15 (18.3)</td>
<td>53 (29.9)</td>
<td></td>
</tr>
<tr>
<td>≥10^9</td>
<td>158 (61.0)</td>
<td>57 (69.5)</td>
<td>101 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Platelet (10^9/L)</td>
<td>203.6±74.4</td>
<td>218.4±61.1</td>
<td>196.8±79.0</td>
<td>0.017</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42 (32–54)</td>
<td>35 (29–47)</td>
<td>44 (34–57)</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>42 (32–56)</td>
<td>45 (21–59)</td>
<td>42 (32–54)</td>
<td>0.315</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.80±0.83</td>
<td>0.89±1.31</td>
<td>0.75±0.45</td>
<td>0.240</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>4.14±0.43</td>
<td>4.32±0.42</td>
<td>4.06±0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>1.09±0.14</td>
<td>1.04±0.10</td>
<td>1.11±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FIB-4 score</td>
<td>1.70±1.11</td>
<td>1.11±0.67</td>
<td>1.96±1.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APRI score</td>
<td>0.62±0.34</td>
<td>0.49±0.24</td>
<td>0.68±0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F0</td>
<td>24 (9.3)</td>
<td>24 (29.3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>61 (23.6)</td>
<td>58 (70.7)</td>
<td>3 (1.7)</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>73 (28.2)</td>
<td>0</td>
<td>73 (41.2)</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>55 (21.2)</td>
<td>0</td>
<td>55 (31.1)</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>46 (17.7)</td>
<td>0</td>
<td>46 (26.0)</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>13 (5.0)</td>
<td>6 (7.3)</td>
<td>7 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>63 (24.3)</td>
<td>36 (43.9)</td>
<td>27 (15.3)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>89 (34.4)</td>
<td>40 (48.8)</td>
<td>49 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>62 (23.9)</td>
<td>0</td>
<td>62 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>32 (12.4)</td>
<td>0</td>
<td>32 (18.1)</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as mean±standard deviation or median (interquartile range) for continuous variables and n (%) for categorical variables. AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; IT, immune-tolerant; INR, international normalized ratio; FIB-4, fibrosis-4; APRI, AST to Platelet Ratio Index;
Proportion of patients with histologic IT phase in comparison with the AASLD and EASL criteria

Among the 259 patients in the serological IT phase, 82 (31.7%) patients were in the histologic IT phase. Patients in the histologic IT phase were younger (36.39 years vs. 42.67 years) on average than those not in the histologic IT phase. The histologic IT phase patients also had comparatively higher HBV DNA (5.5×10^8 IU/mL vs. 2.4×10^8 IU/mL), lower AST (35 U/L vs. 42 U/L), higher albumin, and lower prothrombin time (PT) international normalized ratio (INR) levels (Table 1).

We evaluated the correlation between the current serological criteria provided by AASLD and EASL and the histologic criteria of the IT phase. Out of the enrolled patients, 259 and 100 patients met the serological criteria of the IT phase provided by AASLD and EASL, respectively. Among the 259 patients who satisfied the AASLD criteria, 82 patients (31.7%) were identified to be in the histologic IT phase. Among the 100 patients who satisfied the EASL criteria, 34 patients (34.0%) were identified to be in the histologic IT phase (Supplementary Fig. 2A). In summary, 68.3% and 66.0% of patients adhering to the serological IT phase criteria provided by AASLD and EASL, respectively, were not in the IT phase histologically and may have been in the immune clearance phase.

Similarly, for those patients with ALT within normal limits (≤25 IU for women and ≤35 IU for men), 64.5% and 64.9% of patients adhering to serological IT phase criteria provided by AASLD and EASL, respectively, were not in the IT phase histologically (Supplementary Fig. 2B).

Clinical parameters that can predict the patients who are not likely to be in the histologic IT phase

We investigated useful clinical parameters that can predict patients who are not likely to truly be in the histologic IT phase (Table 2). In a multivariate analysis, factors such as age over 35 years (odds ratio [OR] 1.48, 95% confidence interval

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Female</td>
<td>1.37 (0.65–2.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>1 (ref)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs. ≥35</td>
<td>1.86 (0.61–5.63)</td>
<td>0.274</td>
</tr>
<tr>
<td>vs. ≥40</td>
<td>4.78 (1.31–17.50)</td>
<td>0.018</td>
</tr>
<tr>
<td>vs. ≥45</td>
<td>3.26 (1.53–6.98)</td>
<td>0.002</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.04 (0.91–1.19)</td>
<td>0.570</td>
</tr>
<tr>
<td>HBV DNA (IU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^6–10^7</td>
<td>2.15 (0.61–7.56)</td>
<td>0.231</td>
</tr>
<tr>
<td>10^7–10^8</td>
<td>1.12 (0.52–2.40)</td>
<td>0.778</td>
</tr>
<tr>
<td>&gt;10^8</td>
<td>1 (ref)</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>1.00 (0.99–1.00)</td>
<td>0.240</td>
</tr>
<tr>
<td>AST</td>
<td>1.04 (1.02–1.07)</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>0.99 (0.97–1.01)</td>
<td>0.301</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.17 (0.07–0.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>1.00 (0.67–1.50)</td>
<td>0.995</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[CI] 0.45–4.81, P=0.005), high AST (OR 1.03, 95% CI 1.01–1.06; 
P=0.015) and low albumin level (OR 0.28, 95% CI 0.11–0.73; 
P=0.010) were useful indicators for ruling out histologic IT 
phase. On the other hand, high HBV DNA (≥10^8 IU/mL) or 
gender were not significant factors in predicting the histo-
logic IT phase.

Similarly, we searched for clinical indicators that can predict 
significant fibrosis (≥F2). Age over 35 years, high AST, and low 
albumin were significant indicators and were also useful for 
ruling out the histologic IT phase (Table 3). Though not statis-
tically significant, patients with low HBV DNA levels (10^8–10^7 
IU/mL) had a higher probability of significant fibrosis than patients with high HBV DNA levels.

Factors related with the incidence of liver-
related events (liver cirrhosis, HCC, LT, or death)

During the observation period of 109 months, 192 patients 
(74.1%) switched to the immune-active phase and started 
antiviral therapy. The average time to transition to the im-
mune-active phase was 40.1±48.4 months. During this peri-
od, the development of liver cirrhosis and HCC was evident in 
42 (16.2%) and 17 (6.6%) patients, respectively. Events such as 
LT and death also occurred in one (0.4%) and 21 patients 
(8.1%), respectively. The prediction of long-term prognosis 
was compared between the two IT phase classification 
guidelines. For patients in the histologic IT phase, the inci-
dence of liver-related events was significantly lower than that 
of patients not in the histologic IT phase (log-rank P<0.001, 
Fig. 1A).

We further conducted the Cox regression analysis to identify 
factors related to the incidence of liver-related events (liver 
cirrhosis, HCC, LT, or death). According to our multivariate 
analysis, age (hazard ratio [HR] 1.077, 95% CI 1.045–1.110; 
P<0.0001) and significant fibrosis (F2-F4) (HR 3.650, 95% CI 
1.375–9.694; P=0.009) were closely related to the occurrence 
of liver-related events. On the other hand, histologic inflam-
mation did not show any association with the occurrence of 
liver-related events (Table 4). Similarly, in the Kaplan-Meier 
analysis, the fibrosis stage was associated with the occur-

| Table 3. Multivariate logistic regression predicting significant fibrosis (≥F2) |
|--------------------------|--------------------------|--------------------------|
| Variable | Univariate | Multivariate |
| | | OR (95% CI) | P-value | OR (95% CI) | P-value |
| Sex | | | | |
| Male | 1 (ref) | | | |
| Female | 1.80 (0.98–3.24) | 0.051 | | |
| Age (yr) | | | | |
| <35 | 1.07 (1.04–1.10) | <0.001 | 1.07 (1.04–1.10) | <0.001 |
| vs. ≥35 | 2.12 (0.85–5.31) | 0.106 | 1.79 (0.68–4.69) | 0.235 |
| vs. ≥40 | 4.55 (1.85–11.20) | 0.001 | 4.01 (1.55–10.34) | 0.004 |
| vs. ≥45 | 5.63 (2.93–10.83) | <0.001 | 4.56 (2.27–9.15) | <0.001 |
| Body mass index | 1.06 (0.96–1.17) | 0.288 | | |
| HBV DNA (IU/mL) | | | | |
| 10^8–10^7 | 1.93 (1.01–3.70) | 0.045 | 1.93 (0.95–3.92) | 0.068 |
| 10^8–10^9 | 1.37 (0.61–3.08) | 0.445 | 1.07 (0.44–2.60) | 0.880 |
| >10^9 | 1 (ref) | | 1 (ref) | |
| Platelet | 0.99 (0.99–1.00) | 0.049 | | |
| AST | 1.03 (1.01–1.05) | 0.002 | 1.02 (1.00–1.04) | 0.040 |
| ALT | 0.99 (0.98–1.01) | 0.477 | | |
| Albumin | 0.27 (0.12–0.49) | <0.001 | 0.40 (0.19–0.82) | 0.013 |
| Total bilirubin | 0.86 (0.62–1.19) | 0.359 | | |

OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
Figure 1. Kaplan-Meier curves showing the cumulative incidence of liver-related events (liver cirrhosis, hepatocellular carcinoma, liver transplantation, or death). (A) According to histologic IT phase, (B) according to fibrosis and inflammation grade, (C) according to ALT level. IT, immune-tolerant; ALT, alanine aminotransferase.
rence of liver-related events, whereas inflammation grade showed no evidence of such an association (Fig. 1B).

Further analysis was performed by limiting liver-related events to ‘HCC, LT, and death’, demonstrating similar results and patterns. Age and significant fibrosis were significantly related to the occurrence of HCC, LT, and death. Results of the Kaplan-Meier analysis and Cox regression of sensitivity analysis are presented in Supplementary Table 1 and Supplementary Figure 3.

Importance of age and HBV DNA level for prediction of liver-related events

Various guidelines have mostly used patient age and HBV DNA level to identify the clinical IT phase. We performed a stratified analysis to determine the rate of liver-related events according to age and HBV DNA level (Fig. 2A). Patients under the age of 35 exhibited a very low occurrence rate of liver-related events, regardless of the HBV DNA level (n=1, 1.6%). The incidence of liver-related events was significantly higher in patients over the age of 35 compared to that in patients under the age of 35 (28.7% vs. 1.6%). In particular, the incidence of liver-related events tended to increase (HBV DNA ≥10^6 IU/mL, 25.8%; DNA 10^6–10^7 IU/mL, 29.6%; DNA 10^7–10^8 IU/mL, 37.9%) as the HBV DNA level decreased. Such a trend was further confirmed through the Kaplan-Meier analysis (Fig. 2B).

IT phase patients with normal ALT

We performed a subgroup analysis on patients with normal ALT (≤25 IU for women and ≤35 IU for men). According to histological indication, there was no significant difference in the proportion of patients eligible for treatment between normal ALT and mildly elevated ALT groups (64.5% vs. 69.5%, P=0.531). In addition, the clinical prognosis of the patients with normal ALT eligible for treatment was not as good as that of patients with high ALT (Fig. 1C; log-rank P=0.913). On the other hand, even in patients with normal ALT, age was an

Table 4. Time-dependent covariate Cox regression analysis predicting liver-related events

<table>
<thead>
<tr>
<th>Outcome: liver-related event*</th>
<th>Univariate</th>
<th></th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1 (ref)</td>
<td></td>
<td>1.077 (1.045–1.110)</td>
</tr>
<tr>
<td>Female</td>
<td>1.332 (0.775–2.289)</td>
<td>0.300</td>
<td>1.077 (1.045–1.110)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.088 (1.057–1.120)</td>
<td>&lt;0.001</td>
<td></td>
<td>1.077 (1.045–1.110)</td>
</tr>
<tr>
<td><strong>HBV DNA (IU/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^6–10^7</td>
<td>1.833 (0.917–3.662)</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>10^7–10^8</td>
<td>0.989 (0.530–1.845)</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td>&gt;10^8</td>
<td>1 (ref)</td>
<td></td>
<td>1.077 (1.045–1.110)</td>
</tr>
<tr>
<td><strong>Platelet</strong></td>
<td>0.999 (0.995–1.002)</td>
<td>0.429</td>
<td></td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>0.985 (0.968–1.002)</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>0.766 (0.421–1.394)</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td><strong>Total bilirubin</strong></td>
<td>1.177 (1.005–1.379)</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td><strong>Histologic fibrosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0–F1</td>
<td>1 (ref)</td>
<td></td>
<td>1 (ref)</td>
</tr>
<tr>
<td>F2–F4</td>
<td>5.478 (2.184–13.737)</td>
<td>&lt;0.001</td>
<td>3.650 (1.375–9.694)</td>
</tr>
<tr>
<td><strong>Histologic inflammation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No to minimal</td>
<td>1 (ref)</td>
<td></td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>1.641 (0.972–2.769)</td>
<td>0.064</td>
<td>0.966 (0.556–1.679)</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

*Liver-related event: liver cirrhosis, hepatocellular carcinoma, liver transplantation, or death.
important factor in predicting the presence of the histologic IT phase. Patients aged 35 or older were significantly more likely to be eligible for antiviral treatment than those under 35 years of age (75.0% vs. 45.0%, $P=0.028$).

**DISCUSSION**

Our study demonstrates that the serologically defined IT phase used currently is more inconsistent with the histologic IT phase based on liver biopsy than expected. We also identified additional clinical parameters closely related to the histologic IT phase aside from the previously known HBV DNA, AST, and ALT. This study highlights the necessity for the careful evaluation of the IT profile in adults, the impact of age in predicting fibrosis of F2 or higher, and the importance of liver biopsy.

Ever since the IT phase was first conceptualized by Professor Chu in 1985, it has been classified as a benign phase due to the absence of histologic progression observed during follow-up. However, an increasing number of studies with immunological perspectives have questioned such a notion. A study by Mason et al. found that HBV-specific T cells in the IT phase did not differ from those in the immune active phase. In addition, host genome integration, which is considered the first step in promoting HCC, has already been discovered in the IT phase. According to a similar study involving asymptomatic hepatitis B surface antigen (HBsAg) carrier children from four to nine years old, liver biopsy showed definite histologic changes in the livers of all subjects. In addition to this immunological evidence, clinical findings yield corroborating results of higher HCC incidence in the IT phase than that in the treated immune active phase. Some researchers even argued that the term "IT phase" is a misno-
mer, suggesting the phrase “high replicative, low inflammatory” as a substitution. Similarly, the EASL guideline changed the term “IT phase” to “HBeAg positive infection.”

The definition of the IT phase, even the legitimacy of its very existence, has remained debatable. However, it is crucial to evaluate its definition. Without a rigorous classification of the IT phase, any following conclusion will forever remain invalid. The problem with the IT phase defined by current guidelines, which use clinical and virological parameters, is the overestimation of the incidence of the true IT phase. Moreover, under the current guidelines, it is not possible to distinguish the true histologic IT phase and delayed HBeAg seroconversion.

It is essential to distinguish the two clinically, since the true histologic IT phase demonstrates a good prognosis, whereas delayed HBeAg seroconversion leads to increased risks of HCC and liver cirrhosis. The long-term outcome including HCC in the IT phase has remained debatable among the studies. Most studies have defined the IT phase based on HBV DNA and ALT levels. Previous studies, with the use of pre-existing definitions, are likely to have unintentionally included immune clearance stage patients or delayed HBeAg seroconversion patients, leading to unreliable population samples. Similarly, the sensitivity values of AASLD and EASL criteria were low in our analysis, 16 and 33%, respectively, indicating pre-existing diagnostic criteria cannot accurately identify “true IT phase patients.”

The first finding of our study was in identifying effective indicators that can predict the development of liver-related events, such as liver cirrhosis, HCC, LT, and death. Our study found that it is impossible to determine the prognosis using serological criteria of HBV DNA and ALT. In fact, there have been studies reporting a good prognosis in the biomarker-defined IT phase, but these studies additionally included conditions, such as low Fibrosis-4 (FIB-4) score or age <40 years, in addition to the existing HBV DNA and ALT levels. In the serological IT phase defined by HBV DNA and ALT, one study reported that only 50.3% of patients remained in the IT phase throughout the study period of 63 months.

We concluded that liver biopsy was the only accurate method of evaluating histologic liver fibrosis in IT phase patients. Additionally, histologic fibrosis was associated with long-term prognosis in our analysis. The occurrence rate of liver-related events was 3.65 times higher in the fibrosis of F2 or higher than that in fibrosis of F0 or F1. Therefore, for patients in the suspected IT phase, it is advisable to consider the fibrosis stage regardless of virological markers. Instead of the liver biopsy, transient elastography may be an alternative tool to assess the fibrosis stage, though a potential shortcoming lies in its inability to distinguish fibrosis and moderate-to-severe necroinflammation. Furthermore, the liver stiffness value has been reported to be affected by the degree of inflammation even at a low ALT level. Our analysis of FIB-4 and AST to Platelet Ratio Index (APRI) substantiates such findings, as the proportions of advanced fibrosis (≥F3) in low FIB-4 (<1.45) and in low APRI (<1.0) were 18 and 29%, respectively. Therefore, the clinical usefulness of FIB-4 or APRI is notably low for patients in the IT phase.

The secondary finding of our study was in determining whether a biopsy is necessary for all patients with suspected IT phase. Because liver biopsy, due to its invasive nature, cannot practically be conducted in all patients, it is preferable to conduct biopsy only in patients with advanced fibrosis, the most relevant predictor of long-term prognosis. To reduce the usage of such invasive diagnostic methods, we analyzed clinical factors that can predict fibrosis of F2 or higher. Our results demonstrated that the probability of fibrosis F2 or higher increased significantly by 1.3 times in patients over 35 years, indicating age was the only clinical predictor of fibrosis. Contrary to previous studies, in which age was not considered clinically crucial compared to ALT and HBV DNA, our study suggests otherwise: liver biopsy might be recommended for patients over 35 years of age to evaluate the fibrosis stage histologically.

The tertiary finding of our study is the need for antiviral therapy in the IT phase. Clinical practices recommended by current guidelines cannot accurately identify those who need HBV suppression for HCC prevention among IT phase patients. With the exclusion of such patients receiving treatment based on current guidelines, a missed opportunity to prevent future liver complications inevitably follows. We believe antiviral treatment targeted to the appropriate group will not only hinder liver cirrhosis progression but also prevent HCC. However, it is difficult to use antiviral therapy in every IT phase patient, considering its cost-effectiveness, low adherence in younger patients, long-term side effects, and low virological response in the IT phase. Therefore, to determine the necessity of antiviral therapy, liver biopsy is necessary for patients who have sufficient risk factors for histologic fibrosis of F2 or higher, considering their poor long-term prognosis.
Finally, it is notable that liver-related events tended to increase as HBV DNA decreased in IT phase patients aged over 35 years. Although the strong positive correlation between HBV DNA level and liver-related events such as HCC in hepatitis B patients is well known, an exception is made in IT phase patients, because HBV DNA usually remains very high—above $10^7$ IU/mL—in patients who have never undergone immune clearance.\(^\text{19}\) Therefore, a gradual decrease in HBV DNA level in patients with the IT phase suggests a possibility of immune clearance, necessitating a close clinical observation.

The most important limitation of our study is that our results are derived from retrospective cohort data. First, the time span of our study is too long. During the study period, the diagnostic criteria and guidelines for chronic hepatitis B have been updated and adjusted numerous times; thus, a risk of selection bias remains. In addition, it was not possible to monitor liver-related events according to the specific protocol in all patients. However, ultrasounds, CT scans, or laboratory tests were performed regularly every six months according to the practice guidelines. Also, mortality data in patients with LT were confirmed by the Korean Statistics Promotion Institute (http://stat.or.kr/) and the registry of the Korean Network for Organ Sharing, respectively. Secondly, most Korean hepatitis B patients are known to have genotype C, which displays a delayed e-antigen seroconversion.\(^\text{31}\) Therefore, it is difficult to generalize the 35-year cut-off to other genotypes. A random sampling error in biopsy may exist in our study. Finally, the previous serological criteria were used to include all patients who were presumably in the IT phase, thus we found more patients with advanced liver disease than previously expected. Therefore, we believe that some of the patients might be in the stage of regression of flare in ‘HBeAg positive, immune active infection.’ Regardless, it was helpful to see how many patients in these diverse spectra actually were in the histological IT phase and to further refine the indications for liver biopsy. In addition, similar results were found in subgroup analysis in patients with normal ALT ($\leq 25$ IU for women and $\leq 35$ IU for men).

Future studies on patients in the suspected IT phase need to be conducted to predict the prognosis, using other non-invasive methods to determine fibrosis. Furthermore, the effect of antiviral treatment on the long-term prognosis in patients diagnosed with advanced fibrosis (F2 or higher) through non-invasive methods must be thoroughly scrutinized.

In conclusion, for IT phase patients aged 35 or older who are contemplating treatment options, liver biopsy should be considered without delay instead of waiting for an increase in ALT levels.

**Authors’ contribution**

Study concept and design: Sang Gyune Kim and Yeon Seok Seo. Formal Analysis: Jeong-Ju Yoo. Investigation: All authors. Manuscript writing: Jeong-Ju Yoo. Manuscript review & editing: Sang Gyune Kim and Yeon Seok Seo. Final approval of manuscript: All authors.

**Acknowledgements**

We would like to Jae-Young Kim in Research Factory Inc. (www.rfactory.co.kr) for consulting the statistical analysis.

This research was funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (2021R1G1A1007886), and in part by the Soonchunhyang University Re-search Fund.

**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**


Next-generation sequencing analysis of hepatitis C virus resistance–associated substitutions in direct-acting antiviral failure in South Korea

Kyung-Ah Kim¹*, Sejoon Lee²*, Hye Jung Park³, Eun Sun Jang⁴, Youn Jae Lee⁵, Sung Bum Cho⁶, Young Suk Kim⁷, In Hee Kim⁸, Byung Seok Lee⁹, Woo Jin Chung¹⁰, Sang Hoon Ahn¹¹, Seungtaek Kim¹², and Sook Hyang Jeong⁶

¹Department of Internal Medicine, Inje University Ilsan Paik Hospital, Goyang; ²Department of Precision Medicine Center/Department of Pathology and Translational Medicine, Seoul National University Bundang Hospital, Seongnam; ³Department of Internal Medicine, Institute of Gastroenterology, Yonsei University College of Medicine, Seoul; ⁴Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam; ⁵Department of Internal Medicine, Inje University Busan Paik Hospital, Busan; ⁶Department of Internal Medicine, Chonnam National University Hwasun Hospital, Hwasun; ⁷Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon; ⁸Department of Internal Medicine, Jeonbuk National University Hospital, Jeonju; ⁹Department of Internal Medicine, Chungnam National University Hospital, Daegu; ¹⁰Department of Internal Medicine, Keimyung University School of Medicine, Daegu; ¹¹Zoonotic Virus Laboratory, Institut Pasteur Korea, Seongnam, Korea

Study Highlights

- In Korea, failure of DAAs in patients with chronic hepatitis C occurs mainly in those treated with daclatasvir+asunaprevir for genotype 1b infection or with sofosbuvir+ribavirin for genotype 2 infection. About 50% of DAA-failed patients underwent retreatment with sofosbuvir/velpatasvir/voxilaprevir or glecaprevir/pibrentasvir and achieved a 100% sustained virological response. NS5A RASs at baseline and after virological failure were prevalent in patients with genotype 1b infection and DAA failure. In patients with genotype 2 and DAA failure, RASs were rare both at baseline and after DAA failure. NS5A RASs Y93 and L31 were associated with DAA failure in genotype 1b.
INTRODUCTION

Hepatitis C virus (HCV) infection affects approximately 58 million people worldwide. It is a major cause of liver cirrhosis and hepatocellular carcinoma (HCC).1 The goal of HCV treatment is to achieve a sustained virological response (SVR), defined as undetectable HCV RNA 12 weeks after treatment completion, because it reduces liver-related morbidity and mortality. Direct-acting antivirals (DAA), which target non-structural (NS) proteins essential for HCV replication, have substantially changed the landscape of HCV treatment because they produce an SVR rate greater than 95%.2

Background/Aims: We used next-generation sequencing (NGS) to analyze resistance-associated substitutions (RASs) and retreatment outcomes in patients with chronic hepatitis C virus (HCV) infection who failed direct-acting antiviral agent (DAA) treatment in South Korea.

Methods: Using prospectively collected data from the Korean HCV cohort study, we recruited 36 patients who failed DAA treatment in 10 centers between 2007 and 2020; 29 blood samples were available from 24 patients. RASs were analyzed using NGS.

Results: RASs were analyzed for 13 patients with genotype 1b, 10 with genotype 2, and one with genotype 3a. The unsuccessful DAA regimens were daclatasvir+asunaprevir (n=11), sofosbuvir+ribavirin (n=9), ledipasvir/sofosbuvir (n=3), and glecaprevir/pibrentasvir (n=1). In the patients with genotype 1b, NS3, NS5A, and NS5B RASs were detected in eight, seven, and seven of 10 patients at baseline and in four, six, and two of six patients after DAA failure, respectively. Among the 10 patients with genotype 2, the only baseline RAS was NS5 Y56F, which was detected in one patient. NS5A F28C was detected after DAA failure in a patient with genotype 2 infection who was erroneously treated with daclatasvir+asunaprevir. After retreatment, 16 patients had a 100% sustained virological response rate.

Conclusions: NS3 and NSSA RASs were commonly present at baseline, and there was an increasing trend of NSSA RASs after failed DAA treatment in genotype 1b. However, RASs were rarely present in patients with genotype 2 who were treated with sofosbuvir-ribavirin. Despite baseline or treatment-emergent RASs, retreatment with pan-genotypic DAA was highly successful in Korea, so we encourage active retreatment after unsuccessful DAA treatment. (Clin Mol Hepatol 2023;29:496-509)

Keywords: Hepatitis C virus; Genotype; Drug resistance, viral; Next-generation sequencing

Corresponding author : Seungtaek Kim
Zoonotic Virus Laboratory, Institut Pasteur Korea, 16, Daewangpangyo-ro 712beon-gil, Bundang-gu, Seongnam 13488, Korea
Tel: +82-31-8018-8230, Fax: +82-31-8018-8014, E-mail: seungtaek.kim@ip-korea.org
https://orcid.org/0000-0003-3954-5908

Sook-Hyang Jeong
Department of Internal Medicine, Seoul National University Bundang Hospital, College of Medicine, Seoul National University, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7034, Fax: +82-31-787-4052, E-mail: jsh@snubh.org
https://orcid.org/0000-0002-4916-7990

*These authors contributed equally to this work as co-first authors.

Editor: Hyung Joon Yim, Korea University College of Medicine, Korea
Received : Oct. 28, 2022 / Revised : Feb. 8, 2023 / Accepted : Mar. 1, 2023

Abbreviations:
ASN, asunaprevir; CH, chronic hepatitis; DAA, direct-acting antivirals; DCV, daclatasvir; DSV, dasabuvir; EC50, 50% effective concentration; GLE, glecaprevir; GT, genotype; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LC, liver cirrhosis; LED, ledipasvir; NGS, next-generation sequencing; NS, nonstructural proteins; OMB, ombitasvir; PBMC, peripheral blood mononuclear cells; PIB, pibrentasvir; PTV, paritaprevir; r, ritonavir; RAS, resistance-associated substitution; RBV, ribavirin; SOF, sofosbuvir; ss, single-stranded; SVR, sustained virological response; VEL, velpatasvir; VOXILA, voxilaprevir
HCV is a positive-sense single-stranded (ss) RNA virus that reproduces $10^{10}$ to $10^{12}$ virions per day with an error rate of $10^{-3}$ to $10^{-5}$ mutations per nucleotide per genomic replication. This process can lead to abundant variants, including in the genomic regions targeted by DAA. Resistance-associated substitutions (RASs) are changes in the amino acid sequence of DAA-targeted NS proteins, including NS3A, NS5A, and NS5B. They are commonly present before DAA treatment, but they can also emerge during DAA therapy and can be associated with treatment failure.

In South Korea, the prevalence of anti-HCV antibodies is 0.6–0.71%, and genotypes (GTs) 1b and 2 account for more than 90% of cases. Since the first DAA protocol, daclatasvir+asunaprevir (DCV+ASN), was approved for reimbursement in 2015 for patients with GT 1b in whom mandatory RAS testing showed an absence of NS5A RASs L31 and Y93, it has shown a real-life SVR rate of 94.8–96.3%. Due to cost issues, sofosbuvir/ledipasvir (SOF/LDV) was reimbursed for GT 2 treatment and had a reported SVR rate of 94.2%. After the highly effective pan-genotypic DAA glecaprevir/pibrentasvir (GLE/PIB) was approved in 2018, DCV+ASN, SOF+RBV, and dasabuvir+ombitasvir/paritaprevir/ritonavir (DSV+OMV/PTV/r) were discontinued in practice, though elbasvir/grazoprevir (EBR/GZR) was continued for GT1 patients. SOF/velpatasvir (SOF/VEL) and SOF/VEL/voxilaprevir (SOF/VEL/VOX) were approved in November 2022.

The causes of DAA failure include advanced liver disease, poor compliance, GT3, and the presence of RASs. Prior DAA exposure can select for RASs and attenuate the efficacy of DAA retreatment. Despite the increase of patients with DAA failure, no data are available on RASs or retreatment status in South Korea. Therefore, we used next-generation sequencing (NGS) to analyze the RAS profiles and retreatment outcomes of patients with chronic HCV infection who failed DAA treatment in South Korea.

**MATERIALS AND METHODS**

**Study population**

We analyzed data and blood samples from the South Korean HCV cohort, which prospectively enrolled patients older than 18 years with anti-HCV antibody positivity who voluntarily consented to participate in the study at 10 academic hospitals in South Korea between January 2007 and March 2020. From that population, patients with chronic HCV infection and DAA treatment were the source population for this study (n=1,128). After excluding patients who achieved an SVR after DAA therapy (n=1,028), we included 36 patients in whom DAA failed (Fig. 1). The study protocol was approved by the institutional review board of each hospital (IRB number: 2020-02-020, B-0706-046-002, 2010-01-072, 2012-02-014, 2008-03-009, 2020-02-060, 2020-02-041, 2017-080, 2007-0270, 20016-0345), and written informed consent was

---

**Figure 1.** Study population. HCV, hepatitis C virus; DAA, direct-acting antiviral; N, number; SVR, sustained virological response.
obtained from each enrolled patient before their inclusion in the cohort. This study was conducted according to the tenets of the Declaration of Helsinki.

Data and blood sample collection

Data on laboratory parameters (anti-HCV, serum HCV RNA, and HCV genotype), imaging studies, liver pathology, and transient elastography were collected from medical records upon patient enrollment. At initial enrollment, the participants were classified into three groups: chronic hepatitis, liver cirrhosis, and HCC, as described in previous studies. Patients who underwent successful curative treatment, such as resection or local ablation, and subsequently started DAA treatment were categorized as history of HCC group. The presence of a tumor was defined as a lesion on imaging delineated as HCC; this included individuals with lesions previously treated with chemoembolization who had evidence of radiographic tumor response with tumor necrosis. An active tumor was defined as the presence of arterial enhancement and venous washout on triphasic computed tomography or magnetic resonance imaging. We obtained detailed information about antiviral treatments, which were prescribed at the discretion of the attending physicians.

Patients were prospectively followed every 3–12 months. If patients were lost to follow-up for >6–12 months, the research coordinator at the associated hospital contacted them via phone to confirm their clinical status and encourage a follow-up visit. Follow-up data were entered into the established electronic case report form on the homepage of the Korea Centers for Disease Control, Korean HCV cohort study website. An independent data management team regularly performed quality control.

Blood samples were collected at enrollment beginning in 2014, after obtaining separate consent for research purposes. However, follow-up sampling was not obligatory; therefore, the number of paired samples was small. Blood samples were centrifuged at 2,800 rpm within 2 hours of collection and transferred to a central laboratory within 24 hours under refrigerated conditions. The separated plasma was stored at -70°C.

Next-generation sequencing

HCV RNA was isolated from the plasma using a QIAamp MinElute virus spin kit (QIAGEN, Hilden, Germany). The concentration of ssRNA was calculated using Quant-IT Ribogreen (#R11490; Invitrogen, Waltham, MA, USA). Samples were run on a TapeStation RNA ScreenTape (Agilent, Santa Clara, CA, USA) to assess the integrity of the ssRNA. A library was prepared using 400 ng of ssRNA from each sample and an Illumina TruSeq mRNA sample prep kit (Illumina, Inc., San Diego, CA, USA). The samples were copied into first-strand cDNA using SuperScript II reverse transcriptase (Invitrogen) and random primers, which was followed by second-strand cDNA synthesis using DNA polymerase I and RNase H.

These cDNA fragments were subjected to an end repair process, the addition of a single ‘A’ base, and ligation of the indexing adapters. The products were purified and enriched using polymerase chain reaction (PCR). The libraries were quantified using quantitative PCR (qPCR) according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and were qualified using a TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The indexed libraries were then sequenced by Macrogen Inc. (Seoul, Korea) using the HiSeqXten platform (Illumina).

After sequencing, FastQC (v0.11.5) was used to assess the read quality. Trimmmomatic (v0.36) was used to remove adapter sequences and low-quality reads to reduce bias. DNA sequence data were aligned to the HCV genome reference using the Maximal Exact Match (MEM) algorithm in Burrows-Wheeler Alignment tool (BWA). We sorted the SAM/BAM files and duplication markings using SAMTOOLS v1.9 and SAMBAMBA. We detected single nucleotide variations and small insertions/deletions using mpileup from SAMTOOLS. SNPEFF was used to annotate the identified variants.

FASTQ was purified with VICUNA software to determine the HCV genotype, and each sequencing read was competitively mapped to the HCV subtype reference genome. We counted the number of unique reads of good quality (mapping quality [MAPQ] >50) mapped to each reference and selected the genotype with the most numerous mapped reads.

Statistical analysis

Continuous variables are presented as median (interquartile range) and were compared with Student’s t-test. Categorical variables are presented as numbers (percentages) and were compared using the Chi-square test. An intention-to-
treat analysis was performed assuming that treatment failure occurred when DAA treatment was discontinued for any reason in patients who received at least one dose of a DAA. A per-protocol analysis was performed for patients who completed their treatment on schedule. All statistical analyses were performed using SPSS software version 25 (SPSS Inc., Armonk, NY, USA). All $P$-values were two-sided, and $P<0.05$ was considered statistically significant.

**RESULTS**

**Characteristics of patients with first DAA failure and SVR rate after retreatment**

From October 2012 to May 2020, 1,128 patients were treated with a DAA (Supplementary Table 1). An SVR was achieved in 1,028 of the 1,128 patients, and 64 patients discontinued treatment or were lost to follow-up (SVR rate: 91.1% by intention-to-treat analysis, 96.6% by per-protocol analysis) (Supplementary Table 2). We identified 36 patients (median age of 63 years, 18 males, 4 patients with liver cirrhosis, and 9 pa-

**Table 1.** Characteristics of patients with chronic HCV infection who experienced DAA failure

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Retreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population, n</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Age</td>
<td>63 (56–69)</td>
<td>62 (55–67)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>18/18 (50/50)</td>
<td>10/6 (62.5/37.5)</td>
</tr>
<tr>
<td>Liver disease status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>20 (55.6)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>Compensated cirrhosis</td>
<td>5 (13.9)</td>
<td>3 (6.3)</td>
</tr>
<tr>
<td>Decompensated cirrhosis</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>10 (27.8)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>History of HCC</td>
<td>3 (8.3)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Tumor present</td>
<td>7 (19.4)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Active tumor</td>
<td>4 (11.1)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>HCV Genotype</td>
<td>20/15/1</td>
<td>8/8/0</td>
</tr>
<tr>
<td>1b/2/3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV RNA, log$_{10}$IU/mL</td>
<td>6.0 (5.6–6.5)</td>
<td>6.0 (5.4–6.5)</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>46 (24–81)</td>
<td>32 (19–52)</td>
</tr>
<tr>
<td>Types of failed DAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daclatasvir+asunaprevir</td>
<td>15 (41.7)</td>
<td>5 (28.4)</td>
</tr>
<tr>
<td>Sofosbuvir+ribavirin</td>
<td>13 (36.1)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>Ledipasvir/sofosbuvir</td>
<td>6 (16.7)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>Elbasvir/grazoprevir</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Glecaprevir/pibrentasvir</td>
<td>2 (5.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Previous treatment before DAA therapy</td>
<td>32/4/1 (86.5/10.8/2.7)</td>
<td>13/3/1 (76.5/17.6/5.9)</td>
</tr>
</tbody>
</table>

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

DAA, direct-acting antiviral; HCV, hepatitis C virus; ALT, alanine transferase; IFN, interferon; HCC, hepatocellular carcinoma; CT, computed tomography; MRI, magnetic resonance imaging; DCV, daclatasvir; ASN, asunaprevir; LED, ledipasvir; SOF, sofosbuvir.

$^a$History of HCC was defined as curative treatment, including resection or ablation; $^b$The presence of a tumor was defined as a lesion on imaging delineated as HCC, including individuals with lesions previously treated with radioembolization or chemoembolization who had evidence of a radiographic tumor response with tumor necrosis; $^c$An active tumor was defined as arterial enhancement and venous washout on tri-phasic CT or contrast-enhanced MRI imaging. $^d$A patient who failed first with DCV+ASN and then with LED/SOF treatment.
patients with HCC) with virologic failure of DAA treatment: DCV+ASN in 15, SOF+RBV in 13, LDV/SOF in 6, EBR/GZR in 1, and GLE/PIB in 2. Overall, GTs 1b, 2, and 3 were found in 20, 15, and 1 patient, respectively. Retreatment was performed in 16 patients, mostly with SOF/VOX/VEL or GP, and they had an SVR rate of 100% (Fig. 1). Among those 16 patients, 1 experienced 2 failures, with DCV+ASN and LED/SOF, but finally achieved an SVR with SOF/VOX/VEL (Table 1). SOF/VOX/VEL was not approved in Korea until November 2022, which was after our study period; therefore, it was used in these patients in clinical studies or as individual purchases from foreign pharmacies.

**RAS profiles in patients with GT 1b infection and DAA failure**

The NGS results showed that at least 1 baseline RAS was present in 9 of 10 patients with DCV+ASN or LED/SOF treatment failure; eight had an NS3A RAS, seven had an NS5A RAS, and seven had an NS5B RAS (Fig. 2). NS5A Y93 was the most prevalent RAS at baseline (5/10). Interestingly, the NS5A R30Q RAS was detected in two patients (Table 2).

Posttreatment RASs were analyzed in six patients using samples obtained between 13 and 166 weeks after treatment cessation. All six patients showed at least one post-treatment NS5A RAS: R30 (1/6), L31 (3/6), and Y93 (6/6). All four patients treated with NS3 protease inhibitor showed an NS3 RAS, whereas patients treated with NS5A or NS5B inhibitor did not have NS3 RASs (Table 3). In three patients with pre- and posttreatment samples, one with DCV+ASN treatment failure (#1) had treatment-emergent NS3 Q80R, NS5A L31M/V, and Y93H RASs at 157 weeks after DAA failure. In another patient with DCV+ASN failure (#2), NS3 168A and NS5A L31M RASs emerged, and the frequency of the Y93H RAS increased from 51% at baseline to 100% at 14 weeks after DAA failure. In the third patient, in whom LED/SOF failed (#11), baseline Y93H increased from 34.2% to 100%, but the NS3 RAS Y56F and NS5B RAS 316N were no longer observed by 166 weeks after DAA treatment (Table 4).

Furthermore, 8 of the 20 patients with GT 1b infection and DAA failure were successfully re-treated with DAAs: SOF/VOX/VEL after DCV+ASN failure (n=3), GLE/PB after LED/SOF failure (n=2), DSV+OMV/PTV/r+RBV after LED/SOF failure (n=1), GLE/PB after SOF+RBV failure (n=1, this patient was erroneously diagnosed with GT 2 infection), and SOF/VOX/VEL after failure with first DCV+ASN and then LED/SOF (n=1). Two of those patients with NS5A L31 or Y93 after their first DAA failure were successfully re-treated with SOF/VOX/VEL, and 1

![Figure 2](https://www.e-cmh.org)

**Figure 2.** Prevalence of resistance-associated substitutions in patients with genotype 1b and DAA failure. DAA, direct-acting antiviral; NS, nonstructural protein.
Table 2. Baseline RASs in DAA-failed patients with HCV 1b infection

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/sex</th>
<th>Disease status</th>
<th>Failed DAA</th>
<th>Baseline RAS</th>
<th>Retreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
<td>Age/sex</td>
<td>Disease status</td>
<td>Failed DAA</td>
<td>NS3</td>
<td>NS5A</td>
</tr>
<tr>
<td>1</td>
<td>69/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>S122G (0.97)*</td>
<td>Not detected</td>
</tr>
<tr>
<td>2</td>
<td>64/F</td>
<td>LC</td>
<td>DCV+ASN</td>
<td>Y56F (0.99) S122 (1)</td>
<td>Y93H (0.51)</td>
</tr>
<tr>
<td>3</td>
<td>52/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>4</td>
<td>57/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>S122G (0.99)</td>
<td>R30Q (0.99)</td>
</tr>
<tr>
<td>5</td>
<td>62/F</td>
<td>HCC</td>
<td>DCV+ASN</td>
<td>Y56F (0.98)</td>
<td>R30Q (1)</td>
</tr>
<tr>
<td>6</td>
<td>59/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>S122G (1)</td>
<td>Y93N (0.06) Y93C (0.56)</td>
</tr>
<tr>
<td>7</td>
<td>63/M</td>
<td>LC</td>
<td>DCV+ASN</td>
<td>S122G (0.96)</td>
<td>Y93H (1)</td>
</tr>
<tr>
<td>8</td>
<td>51/M</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>S122G (1)</td>
<td>Not detected</td>
</tr>
<tr>
<td>11</td>
<td>66/M</td>
<td>CH</td>
<td>LED/SOF</td>
<td>Y56F (0.19)</td>
<td>Y93H (0.34)</td>
</tr>
<tr>
<td>12</td>
<td>63/M</td>
<td>HCC</td>
<td>LED/SOF</td>
<td>Not detected</td>
<td>Y93H (1)</td>
</tr>
</tbody>
</table>

ASN, asunaprevir; CH, chronic hepatitis; DAA, direct-acting antivirals; HCV, hepatitis C virus; DCV, daclatasvir; DSV, dasabuvir; GLE, glecaprevir; HCC, hepatocellular carcinoma; LC, liver cirrhosis; LED, ledipasvir; OMB, ombitasvir; PIB, pibrentasvir; PTV, paritaprevir; r, ritonavir; RAS, resistance-associated substitution; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virological response; VEL, velpatasvir; VOX, voxilaprevir; w, weeks. Figures in parentheses are the frequencies of substitution.
### Table 3. Post-treatment RASs in DAA-failed patients with HCV 1b infection

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/sex</th>
<th>Disease status</th>
<th>Failed DAA</th>
<th>Time of RAS test</th>
<th>Posttreatment RAS</th>
<th>Retreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS3</td>
<td>NS5A</td>
</tr>
<tr>
<td>1</td>
<td>69/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>157 w after ETR</td>
<td>Q80R (0.28)*</td>
<td>L31M (0.99)</td>
</tr>
<tr>
<td>2</td>
<td>64/F</td>
<td>LC</td>
<td>DCV+ASN</td>
<td>14 w after ETR</td>
<td>Y56F (0.99)</td>
<td>L31M/V (1)</td>
</tr>
<tr>
<td>9</td>
<td>67/F</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>170 w after ETR</td>
<td>Y56F (0.99)</td>
<td>L31M (1)</td>
</tr>
<tr>
<td>10</td>
<td>46/M</td>
<td>CH</td>
<td>DCV+ASN</td>
<td>25 w after ETR</td>
<td>Y56F (1)</td>
<td>L31M (1)</td>
</tr>
<tr>
<td>11</td>
<td>66/M</td>
<td>CH</td>
<td>LED/SOF</td>
<td>166 w after ETR</td>
<td>Not detected</td>
<td>Y93H (1)</td>
</tr>
<tr>
<td>13</td>
<td>48/M</td>
<td>HCC</td>
<td>SOF+RBV†</td>
<td>128 w after ETR</td>
<td>Not detected</td>
<td>R30Q (1)</td>
</tr>
</tbody>
</table>

ASN, asunaprevir; CH, chronic hepatitis; DAA, direct-acting antivirals; DCV, daclatasvir; ETR, end of treatment; GLE, glecaprevir; HCC, hepatocellular carcinoma; LC, liver cirrhosis; LED, ledipasvir; RAS, resistance-associated substitution; SOF, sofosbuvir; SVR, sustained virological response; VEL, velpatasvir; VOX, voxilaprevir; w, weeks.

*Figures in parentheses are the frequencies of substitution; †This patient was misclassified as genotype 2.

### Table 4. Baseline and posttreatment RASs in DAA-failed patients with paired samples

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/sex</th>
<th>Disease status</th>
<th>Genotype</th>
<th>Failed DAA</th>
<th>Baseline RAS</th>
<th>Posttreatment RAS</th>
<th>Retreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS3</td>
<td>NS5A</td>
<td>NS5B</td>
</tr>
<tr>
<td>1</td>
<td>69/F</td>
<td>CH</td>
<td>1b</td>
<td>DCV+ASN</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Q80R (0.28)</td>
</tr>
<tr>
<td>2</td>
<td>64/F</td>
<td>LC</td>
<td>1b</td>
<td>DCV+ASN</td>
<td>Y56F (0.99)</td>
<td>Y93H (0.51)</td>
<td>Y56F (0.99)</td>
</tr>
<tr>
<td>11</td>
<td>66/M</td>
<td>CH</td>
<td>1b</td>
<td>LED/SOF</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>14</td>
<td>58/F</td>
<td>CH</td>
<td>2a/2c</td>
<td>SOF+RBV</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>25</td>
<td>48/F</td>
<td>CH</td>
<td>2a</td>
<td>DCV+ASN†</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

ASN, asunaprevir; CH, chronic hepatitis; DAA, direct-acting antivirals; DCV, daclatasvir; GLE, glecaprevir; LC, liver cirrhosis; LED, ledipasvir; RAS, resistance-associated substitution; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virological response; VEL, velpatasvir; VOX, voxilaprevir; w, weeks.

*Figures in parentheses are the frequencies of substitution; †This patient was misclassified as genotype 1b.
RAS profiles in patients with GT 2 infection and DAA failure

The types of DAA failure in the 15 patients with GT 2 were as follows: SOF+RBV in 12, LED/SOF in one, GLE/PIB in one, and DCV+ASN in one. RASs were analyzed in 12 samples from 10 patients.

Six patients were tested for baseline RASs, including four with SOF+RBV failure, one with LED/SOF failure, and one with DCV+ASN failure. Only one of the six tested patients showed the NS3 Y56F RAS; however, neither an NS5A RAS nor NS5B RAS was found at baseline in these patients (Table 5). Post-treatment, the NS5B RAS was found in five patients who experienced SOF+RBV failure, and only one patient, who was misidentified as having a GT 1b infection, showed emergence of the NS5A R28C RAS after DCV+ASN failure (Table 6).

Among the 12 patients with GT 2 infection and SOF+RBV failure, 7 were successfully re-treated (6 with GLE/PIB and one with pegylated interferon+RBV). The patient erroneously given DCN+ASN was successfully re-treated with EBR/GZR+RBV.

RAS profile in the patient with GT 3 infection and DAA failure

The one patient with compensated cirrhosis and GT 3a infection was treated with GLE/PIB for 12 weeks but experienced virological failure. The baseline RAS was analyzed for this patient, and the NSSA RAS A30K was detected at a frequency of 100%. This patient has not yet been re-treated.

DISCUSSION

This study demonstrated the RAS features and retreatment outcomes of 36 patients with chronic HCV infection who experienced DAA failure in South Korea. Among the 10 patients with GT 1b, baseline RASs in NS3, NS5A, and NS5B were detected in eight, seven, and seven, respectively. After DAA failure, RASs in NS3, NS5A, and NS5B were detected in four, six, and two of six patients, respectively. However, among patients with GT 2, the only RAS detected at baseline was NS3...
Y56F in one patient; after DAA failure, the NSSA F28C RAS was found in one patient who was erroneously treated with DCV+ASN. Among all 36 patients in our sample, 16 were retreated, with an SVR rate of 100%; therefore, active retreatment following a first DAA failure is recommended.

In the initial phase of DAA introduction in Korea, most patients with GT 1b infection were treated with DCV+ASN or LED/SOF, whereas those with GT 2 infection were treated with SOF+RBV. Although the SVR rates of genotype-specific DAAs are as high as 94.2–96.2%, 11,12 36 patients with virological failure on DAA were identified in this study. Baseline NSSA RASs significantly lowered the SVR rate in patients treated with DCV+ASN (65.4% vs. 94.3%) 20 in a previous clinical trial. Therefore, before 2017, a negative Sanger sequencing test for the NSSA RASs L31 and Y93 was mandatory for reimbursement of the DCV+ASN regimen in South Korea. 21 Among the 13 patients without a baseline RAS, as shown by Sanger sequencing, who were treated with DCV+ASV, NGS showed the presence of the Y93H RAS in 2. One patient had the Y93C/N RAS (56%/6%), and the other had the Y93H RAS (51.5%). Although the detection limit of Sanger sequencing is known to be 15–20%, a variant at a relatively low frequency (51.5%) could be missed by Sanger sequencing but detected with NGS.

Among the patients with GT 1b infection, the Y93 RAS was detected in 50% and 100% at baseline and after virological failure, respectively, whereas the L31 RAS was detected in 50% of patients after virological failure. Both the L31M and Y93H RASs significantly increased the 50% effective concentration (EC_{50}) of DCV in GT 1b in vitro and exhibited synergism, showing >1,000-fold changes in EC_{50}. 22 Considering that the baseline prevalence of the NSSA RASs L31 and Y93 in Korean patients with GT 1b infection was reported to be 5.6% and 15.5%, respectively, 23 they were likely to be associated with virological failure in Korean patients with GT 1b infection. Moreover, an international cohort study reported that NSSA RASs, including L31 and Y93, increased from 11% at baseline to up to 73% after failure with NSSA inhibitors. 24 The R30Q RAS by itself was not reported to be associated with DCV resistance, but it did increase the EC_{50} of DCV by 31,000–37,000-fold when it co-presented with L31M and Y93. 23 In our study, treatment-emergent NSSA RASs persisted at a high frequency (99%) for up to 157 weeks, which is compatible with previous results indicating that NSSA RASs persisted for 48 weeks posttreatment, whereas NS3 RASs returned to the

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Disease status</th>
<th>DAA</th>
<th>Time of RAS test</th>
<th>RAS</th>
<th>Retreatment</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>56/M</td>
<td>CH</td>
<td>SOF+RBV</td>
<td>76 w after ETR</td>
<td>Not detected</td>
<td>ELB/GRZ+RBV</td>
<td>12 w</td>
</tr>
<tr>
<td>19</td>
<td>76/M</td>
<td>CH</td>
<td>SOF+RBV</td>
<td>39 w after ETR</td>
<td>Not detected</td>
<td>ELB/GRZ+RBV</td>
<td>12 w</td>
</tr>
<tr>
<td>20</td>
<td>69/M</td>
<td>HCC</td>
<td>SOF+RBV</td>
<td>30 w after ETR</td>
<td>Not detected</td>
<td>ELB/GRZ+RBV</td>
<td>12 w</td>
</tr>
<tr>
<td>21</td>
<td>69/M</td>
<td>CH</td>
<td>DCV+ASN*</td>
<td>96 w after ETR</td>
<td>Not detected</td>
<td>ELB/GRZ+RBV</td>
<td>12 w</td>
</tr>
<tr>
<td>23</td>
<td>48/F</td>
<td>CH</td>
<td>CH</td>
<td>48 w after ETR</td>
<td>Not detected</td>
<td>ELB/GRZ+RBV</td>
<td>12 w</td>
</tr>
</tbody>
</table>

*This patient was misclassified as genotype 1b; †Figures in parentheses are the frequencies of substitution.
wild type within 16 weeks after cessation, according to a
direct sequencing analysis.\textsuperscript{25}

\textbf{NS5 RASs Q80K/R and D168A/C/E/G/H/Y/Y were associated
with resistance to asunaprevir in vitro and in vivo}, but the role
of the S122G RAS in asunaprevir failure was unclear.\textsuperscript{22} Y56F
was associated with resistance to grazoprevir.\textsuperscript{23} The
prevalence of natural NS3 RASs Q80, D168, S122G, and Y56F was
reported to be 3.9%, 0.7%, 9.34%, and 26%, respectively.\textsuperscript{24} In
the present study, treatment-emergent Q80R and D168A
RASs were associated with DCV+ASN failure and were
detected at frequencies of 0.28% and 17% at 157 and 14 weeks
after treatment cessation, respectively. Considering that
almost all NS3 RASs returned to wild type after DAA cessa-
tion,\textsuperscript{25} these NS3 RASs might be present at higher frequen-
cies immediately after DAA cessation.

\textbf{NS5B RASs C316N and S556G in the GT 1b patients in our
study population might be naturally occurring for non-nucle-
otide inhibitors. The prevalence of naturally occurring NS5B
RASs in GT 1b was reported to be 12–25%, and the C316N
RAS was the most prevalent.}\textsuperscript{26,27} Therefore, our result is
consistent with those in previous reports.

When patients experience failure with NSSA or NS3 inhibi-
tors, treatment with SOF/VOX/VEL is recommended.\textsuperscript{28} How-
ever, DCV+ASN has mainly been used in East Asians, and few
data are available on retreatment of patients with SOF/VOX/
VEL. Although our patient population was small, we showed
that treatment with SOF/VOX/VEL was highly effective for GT
1b HCV after DCV+ASN failure. In a Japanese study that evalu-
ated the efficacy of LED/SOF treatment in patients who
failed DCV+ASN treatment, the SVR rate was 86.7%, and the
presence of cirrhosis and both NSSA L31 and Y93 RASs were
poor response factors. In this study, a patient with a baseline
Y93H RAS and DCV+ASN treatment failure who also failed
LED/SOF+RBV and had treatment-enriched Y93H and treat-
ment-emergent L31M/V was successfully treated with SOF/
VOX/VEL.

Although GLE/PIB is not recommended for patients who fail
an NSSA inhibitor-containing regimen, two patients with
LED/SOF failure and NSSA Y93H RASs were treated with GLE/
PIB and achieved an SVR. In GT 1b patients, NSSA Y93 and L31
RASs did not influence the treatment outcome with pipren-
tasvir.\textsuperscript{22} In a study conducted in Japan, the SVR rate with GLE/
PIB in patients with GT 1b who experienced failure with
DCV+ASN or LED/SOF was 87.5%, and multiple NSSA RASs
were detected in patients with GLE/PIB failure.\textsuperscript{29} Therefore,
SOF/VOX/VEL should be the retreatment regimen in patients
with DCV+ASN failure.

The NSSB S282T RAS in GT 2a was associated with de-
creased susceptibility to sofosbuvir in vitro.\textsuperscript{30} Although the
emergence of NSSB S282T\textsuperscript{31} and L159F\textsuperscript{32} was reported in
patients with GT 2b infection who experienced virologic failure
with SOF+RBV, selection of sofosbuvir-resistant HCV is very
rare and is associated with a significant reduction in viral fit-
ness.\textsuperscript{33} Therefore, virologic failure of SOF+RBV in patients
with GT 2 infection might be associated not with RASs, but
with other factors such as innate inadequacy of this regimen
for GT 2, liver disease severity, ribavirin dosage, or medication
adherence.\textsuperscript{34} In this study, we found no baseline or treat-
ment-emergent NSSB RASs in patients with GT 2 infection
who experienced failure with SOF+RBV. The proportion of
HCC was higher in patients with SOF+RBV failure than in
those with an SVR (30.8% vs. 9.8%), but the proportion of ac-
tive HCC did not differ, probably due to the small number of
active HCC patients. A study reported that active HCC was
associated with DAA failure,\textsuperscript{36} so HCC might be one of the rea-
sons for SOF+RBV failure. However, the time from the end of
treatment to RAS testing ranged from 9–95 weeks, so the
possibility that treatment-emergent RASs reverted to wild
type could not be excluded. Treatment with GLE/PIB was
highly effective in patients with GT 2 infection who experi-
enced failure with SOF+RBV, showing a 100% SVR rate, and is
currently recommended for patients with sofosbuvir failure.\textsuperscript{35}
In our study, GLE/PIB was also highly effective in patients
with GT 2 infection and failure with SOF+RBV therapy.

The prevalence of GT 3 is very low (<1% in Korea),\textsuperscript{36} and it is
the most difficult genotype to treat because of the high fre-
quency of RASs. Current guidelines recommend 8–12 weeks
of GLE/PIB treatment for treatment-naive GT 3 infections
with compensated cirrhosis because of its 95% SVR rate.\textsuperscript{28,37}
In this study, a treatment-naive patient with GT 3a infection
and compensated cirrhosis experienced failure after 12
weeks of GLE/PIB therapy, and the NSSA A30K RAS was de-
tected at a 100% frequency in that patient at baseline. With
GT 3a, the NSSA RAS A30K occurs naturally with a frequency of
6% and does not decrease pibrentasvir sensitivity by itself,
but it does lower sensitivity when Y93H is also present.\textsuperscript{38}
Therefore, it is unclear whether the NSSA A30K RAS was
associated with GLE/PIB failure in this patient.

Most laboratories currently use Sanger sequencing as the
gold standard for RAS tests; however, NGS offers potential
advantages in terms of throughput, accuracy, and detection of low-frequency variants. Recent studies have shown that the results of NGS are highly concordant with those of Sanger sequencing. However, NGS requires higher viral loads (4.5 log_{10} IU/mL) than Sanger sequencing (1,000 IU/mL) for RAS detection. In our study, NGS corrected genotyping errors in 2 patients. NGS has been reported to have 96.1% specificity in determining the HCV genotype, and it is useful for distinguishing mixed infections.

Our study has several limitations. First, the small number of patients with DAA failure is insufficient for the study results to be generalizable. Second, RASs were not analyzed both at baseline and after virological failure for all patients; therefore, it was difficult to interpret whether the RASs detected after DAA failure were present at baseline or emerged after treatment failure. Third, the time points for the RAS analyses after virologic failure were not uniform, and RASs can be gradually replaced by the wild type, especially NS3 RASs. Fourth, we did not analyze RASs for the SVR group and thus cannot evaluate how the RASs affected treatment outcomes by directly comparing the SVR group and DAA-failure group. However, in other studies, the prevalence of natural RAS in treatment-naïve patients was much lower than in the patients with virological failure in our study. Fifth, because fewer than 50% of the patients with DAA failure were re-treated with a DAA, the number of re-treated patients was too small for a suitable statistical analysis; therefore, the retreatment outcomes of patients with DAA failure could be biased. The low retreatment rate is partly because SOF/VEL/VOX is currently commercially unavailable, and Korea did not have an option for NSSA inhibitor failure during the study period. Since rescue therapy with SOF/VEL/VOX has been reimbursed in Korea since November 2022, the efficacy of rescue therapy can be further elucidated. However, the retreatment rate of patients with GT 2 is also unsatisfactory, probably because of the absence of reimbursement for retreatment or cost issues for second-line treatment. Therefore, efforts should be made to improve the retreatment rate and therapeutic regimens of patients with DAA failure.

In conclusion, our study revealed that DAA failure occurs mainly in patients treated with DCV+ASN for GT 1b infection and those treated with SOF+RBV for GT 2 infection; approximately half of DAA-failed patients underwent retreatment with SOF/VEL/VOX or GP, and they had a 100% SVR irrespective of the presence of baseline or posttreatment RASs. NSSA RASs at baseline and after virological failure were prevalent in patients with GT 1b infection and DAA failure. In contrast, RASs in patients with GT 2 and DAA failure were rare both at baseline and after DAA failure. NSSA RASs Y93 and L31 were associated with DAA failure in GT 1b. Further studies are required to assess treatment outcomes and the factors of treatment failure.

Authors’ contribution

Kyung-Ah Kim: Study concept and design; acquisition of data; analysis and interpretation of data; drafting manuscript; critical revision of the manuscript for important intellectual content; obtained funding; study supervision. Sejoon Lee: Study concept and design; acquisition of data; analysis and interpretation of data; drafting manuscript; critical revision of the manuscript for important intellectual content. Hye Jung Park: Laboratory experiment. Eun Sun Jang, Youn Jae Lee, Sung Bum Cho, Young Suk Kim, In Hee Kim, Byung Seok Lee, Woo Jin Chung: Acquisition of data, critical revision of the manuscript for important intellectual content. Seungtaek Kim: Study concept and design; interpretation of data; laboratory experiment; critical revision of the manuscript for important intellectual content; study supervision. Sook-Hyang Jeong: Study concept and design; interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision. All authors approved the final version of the manuscript.

Acknowledgements

This study was supported by a research grant (#2020-E5105-02) from the National Institute of Infectious Disease, Korea Disease Control and Prevention Agency and a grant for the Chronic Infectious Disease Cohort Study (Korea HCV Cohort Study, No. 2020-E5104-02) from the National Institute of Infectious Disease, Korea Disease Control and Prevention Agency.

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


Letter to the Editor

Letter regarding “Long-term prognosis and the need for histologic assessment of chronic hepatitis B in the serological immune-tolerant phase”

Chia-Ming Chu and Yun-Fan Liaw
Liver Research Unit, Chang Gung Memorial Hospital, Taipei, Taiwan

Keywords: Chronic hepatitis B; Immune tolerance; Age; Alanine aminotransferase; Hepatitis B virus

Dear Editor,

In a recent issue of this journal, Yoo et al.\(^1\) reported a high frequency of significant inflammation and fibrosis in immunotolerant (IT) chronic hepatitis B patients and highlighted the need of liver biopsy for these patients. There are a few points that merit clarification and further discussion.

First, the average age of IT patients was quite high (42.7±12.5 years). The median age of IT patients ranged from 29 to 31 years in 4 previous studies.\(^2-5\) Our earliest study in 1985, when we coined the term of IT, showed that the average age of 64 hepatitis B e antigen (HBeAg)-positive patients with minimal histological changes was 25±5 years.\(^6\) In a later study, the age of 240 HBeAg-positive patients with persistently normal alanine aminotransferase (ALT) was 28±6 years.\(^7\) One possible explanation could be that genotype C hepatitis B virus (HBV) predominates in Korea and that HBeAg seroconversion is significantly delayed in genotype C than genotype B infection (mean age of HBeAg seroconversion: 36±4±8.6 vs. 31.8±±7.0 years).\(^8\) In addition, there appears to be a high selection bias, as patient enrollment in this study was based on biopsy which is strongly recommended in elderly patients to exclude significant disease.\(^9\)

Second, there is discordance between ALT and histological activity. It remained unclear why ALT levels were not significantly different between histologic IT and non-IT patients. On the other hand, only 35% of the patients with normal ALT (≤35 U/L for men and ≤25 U/L for women according to American Association for the Study of Liver Diseases [AASLD] 2018 guidelines)\(^9\) were proved to be histologic IT patients. Many of these patients appear to be in the immune active phase with normal ALT at remission rather than truly in the IT phase. Diagnosis of IT in this study requiring at least two ALT measurements >3 months apart appears to be insufficient.

Third, the majority of IT patients demonstrated significant inflammation or fibrosis. Notably, the patients in this study were relatively older, with relatively lower HBV DNA (≥10^6 IU/mL vs. ≥10^7 IU/mL) and higher ALT (≤60 U/L vs. ≤40 U/L). Two earlier histologic studies including 57 and 40 patients with median age of 29 and 31 years, respectively, HBV DNA >10^7 copy/mL and normal ALT revealed only mild disease in all and no patients had significant fibrosis.\(^3,5\) In another study

Corresponding author: Chia-Ming Chu
Liver Research Unit, Chang Gung Memorial Hospital, 199, Tung Hwa North Road, Taipei, 105 Taiwan
Tel: +886-3-3281200 ext. 8107, Fax: +886-3-3272236, E-mail: chiamingchu@yahoo.com.tw
https://orcid.org/0000-0003-0917-0439

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

Received: Jan. 19, 2023 / Revised: Jan. 20, 2023 / Accepted: Jan. 20, 2023
including 40 patients with normal ALT and HBV DNA of 8.14 (4.83–10.96) log₈ IU/mL, significant inflammation and fibrosis was noted in 2 and 0 of 17 patients with ALT ≤0.5xupper limit of normal (ULN), and in 7 and 4 of 23 patients with ALT 0.5–1xULN. A large series study from China recruited 202 stringently defined IT patients with HBV DNA ≥10⁷ IU/mL and ALT ≤40 U/L, according to European Association for the Study of the Liver (EASL) 2017 guidelines, for at least 2 years. Significant inflammation and fibrosis were extremely rare (2% and 0%) in 97 patients with low-normal ALT (≤30 U/L for men and ≤19 U/L for women), regardless of patient age, but much frequent (39% and 10%) in 105 patients with high-normal ALT (31–40 U/L for men and 20–40 U/L for women). Among the latter, the severity of histological activity correlated with patient age. These data from “genuine IT” suggest that it seems appropriate to use the EASL 2017 guidelines to define IT phase, but for patients over 40 years old, it is better to use low-normal ALT.

Fourth, there was a high incidence of liver-related events in IT patients; cumulative rates at 15-years follow-up were 15% and 45% for histologic IT and non-IT patients, respectively. However, chronic HBV infection is a dynamic process that undergoes transition through various phases of disease activity. Patients should be censored at the time of phase transition, otherwise their results will be misleading.

Finally, the authors suggested to treat IT patients with significant fibrosis. Sixty-seven percent of patients had significant fibrosis, implying the need for systematic histologic evaluation in all. However, liver biopsy is an invasive procedure with potential complication. Recently, antiviral therapy is recommended for IT patients over age of 30 or 40 without the need of histological assessment. Studies from Korea have shown an extremely low or negligible risk of hepatocellular carcinoma (HCC) in IT patients with HBV DNA ≥10⁷ IU/mL, HBV DNA ≥10⁶ IU/mL and age <40, and FIB-4 index <1.45. Therefore, it is recommended to use strict clinical criteria to define IT phase, i.e., HBV DNA ≥10⁷ IU/mL and ALT ≤40 IU/L every 3 months for at least 1 year. Antiviral treatment can be limited to those over 40 years old only if they have high-normal ALT, significant fibrosis as seen using non-invasive serum fibrosis markers or Fibroscan, or family history of HCC.

In summary, strict clinical criteria are needed to define IT phase of chronic hepatitis B infection to avoid clinical confusion and unnecessary liver biopsy or treatment.

Authors’ contributions

CM Chu: Conception and design of the letter; Drafting of the manuscript; Approval of the final version of the manuscript. YF Liaw: Design of the letter; Critical revision of the manuscript; Approval of the final version of the manuscript.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

6. Chu CM, Liaw YF, Sheen IS, Chen TJ. Correlation of age with the status of hepatitis B virus replication and histological changes

Abbreviations:

AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; EASL, European Association for the Study of the Liver; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IT, immune tolerance; ULN, upper limit of normal

8. Chu CM, Liaw YF. Genotype C hepatitis B virus infection is associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B: a longitudinal study of hepatitis B e antigen-positive patients with normal aminotransferase levels at baseline. J Hepatol 2005;43:411-417.
Dear Editor,

We would like to thank Chu and Liaw1 for their interest in our paper2 and for providing valuable insights into the immune tolerant (IT) phase. We acknowledge the potential for selection bias in our study due to the relatively high average age of the patients included. We included all patients with the current IT criteria to highlight the wide range of the IT phase. If limited to patients younger than 30 years of age, four out of 51 patients had advanced fibrosis. Out of those four patients, hepatocellular carcinoma occurred in only one patient with severe fatty liver. Likewise, many IT phase patients who meet the current guidelines’ criteria often require treatment in real practice.

There is a general agreement that IT phase patients with significant fibrosis should receive treatment.3,4 Although our study included a large number of such patients, we do not believe that a liver biopsy is necessary for all IT phase patients. The challenge is to accurately identify those with significant fibrosis without a biopsy. There are many diagnostic tools for non-invasive fibrosis, such as transient elastography (TE) or magnetic resonance elastography, and these can be used as secondary tools for accurate diagnosis of the IT phase and excluding significant liver fibrosis.5,6 Although seroconversion can be delayed in genotype C patients (commonly found in South Korea), it is usually accompanied by significant fibrosis in patients over 35 years of age.7 However, the current IT phase diagnosis guidelines only use serological criteria. Our data showed that serum alanine aminotransferase (ALT) levels alone do not fully reflect the histological activity of IT phase. In the same vein, previous studies8–10 have found that ALT levels do not indicate the actual inflammation in the liver due to the following reasons: i) ALT elevation is associated with the location of inflammation,11 and ii) the changes in ALT levels occur faster than the changes in histology.12 One of the alternatives, as mentioned1 by

Keywords: Chronic hepatitis B; Immune tolerance; Histology

Corresponding author: Sang Gyune Kim
Department of Gastroenterology and Hepatology, Digestive Research Center and Liver Clinic Soonchunhyang University Bucheon Hospital, 170 Jomaru-ro, Wonn-mu, Bucheon 14584, Korea
Tel: +82-32-621-5215, Fax: +82-32-621-6079, E-mail: mcnulty@schmc.ac.kr
https://orcid.org/0000-0001-8694-777X

Editor: Seung Up Kim, Yonsei University College of Medicine, Korea
Received: Feb. 6, 2023 / Revised: Feb. 9, 2023 / Accepted: Feb. 9, 2023

Copyright © 2023 by Korean Association for the Study of the Liver
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
the authors, is that different standards for normal ALT levels might be applied according to age.

Nevertheless, we completely agree with the authors’ view of the IT phase patients, and we also believe that ALT levels should be monitored every 3–6 months to detect transitions from the IT phase to the immune active phase. Although there are many diagnostic tools for fibrosis and steatosis with the recent development of technology, there is no powerful diagnostic tool for inflammation other than a liver biopsy. In this case, TE can provide information about inflammation as well as fibrosis, but the measured stiffness value should be used with caution. Our research team has found that the liver stiffness value is related to both histologic inflammation and fibrosis in patients with ALT levels less than 200 U/L.

In conclusion, our study highlights the concern that the current IT phase guidelines may delay treatment for actually non-IT phase patients with hepatitis B. As mentioned by Chu and Liaw, we fully support the opinion that the HBV DNA cut-off value in the IT phase should be set very high, similar to the EASL guideline, and that the ALT criteria should be adjusted according to age. We hope that future IT phase guidelines will include age criteria and non-invasive diagnostic technologies for accurate fibrosis and/or inflammation diagnosis.

Authors’ contribution
Writing manuscript: Jeong-Ju Yoo and Sang Gyune Kim, Supervision: Sang Gyune Kim.

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

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES

Abbreviations:
IT, immune tolerant; TE, transient elastography; MRE, magnetic resonance elastography; ALT, alanine aminotransferase
Snapshot

Systemic therapy in advanced hepatocellular carcinoma

Joseph C. Ahn¹, Nguyen H. Tran², and Ju Dong Yang³,⁴,⁵

¹Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; ²Department of Medical Oncology, Mayo Clinic, Rochester, MN; ³Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA; ⁴Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA; ⁵Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Corresponding author : 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

Future Directions

- New combinations of immunotherapy and molecularly targeted agents
- New combinations of systemic therapy and locoregional therapy
- Drugs targeting new pathways
- Novel biomarkers to predict response to specific therapy
- Systemic therapy for early, intermediate stage HCC.
While advanced hepatocellular carcinoma (HCC) is an incurable disease, decades of research and clinical trials have led to substantial progress in treatment for advanced HCC.\(^1\) In the 20th century, patients with advanced HCC were treated with conventional chemotherapy which offered little to no benefit with significant adverse effects.\(^2\) The first breakthrough came with the molecularly targeted agents in the family of oral multi-tyrosine kinase inhibitors. In 2007, the multi-target kinase inhibitor sorafenib became the first-line systemic therapy for advanced HCC.\(^3\) Since 2017, multiple positive phase III clinical trials have led to the approval of several additional molecularly targeted agents—lenvatinib\(^4\) as a first-line, and regorafenib,\(^5\) cabozantinib,\(^6\) and ramucirumab\(^7\) as second-line options. Additional multikinase inhibitors such as donafenib\(^8\) and apatinib\(^9\) have shown promising results in phase III trials conducted in China.

A dramatic therapeutic paradigm shift was driven by the advent of immune checkpoint inhibitors targeting programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). The first studied immunotherapy agents in HCC were the anti-PD-1 antibodies, nivolumab and pembrolizumab, which demonstrated clinical benefit and pembrolizumab has received accelerated approval as second-line agents following sorafenib.\(^10,11\) More recently, tislelizumab (anti-PD-1 monoclonal antibody) demonstrated non-inferior overall survival (OS) compared to sorafenib (median OS: 15.9 months vs. 14.1 months; stratified hazard ratio [HR] 0.85 [95.003% CI 0.712–1.019]) in first-line setting phase 3 trial.\(^12\)

In 2020, the results of the landmark IMbrave 150 study led to the largest change in the treatment landscape of advanced HCC.\(^13\,14\) In this global phase III trial of patients with untreated unresectable HCC, the combination of atezolizumab (anti-PD-L1 antibody) and bevacizumab (anti-vascular endothelial growth factor antibody) was significantly superior to sorafenib with an improved median overall survival (19.2 months vs. 13.4 months, HR 0.58, 95% CI 0.42–0.79) and comparable rates of grade 3 or 4 adverse events (43% vs. 46%).\(^15,16\) This has quickly led to the adoption of atezolizumab-bevacizumab as the standard first-line systemic therapy.\(^15\,17\)

Immunotherapy doublets have shown promising results.\(^2\) The addition of an anti-CTLA-4 antibody to an anti-PD-1 or PD-L1 antibody significantly enhances the antitumor response by increasing the intratumoral concentration of T lymphocytes.\(^18\) The combination of nivolumab and CTLA-4 inhibitor ipilimumab was approved as second-line therapy based on the CheckMate 040 study that showed manageable safety, promising response rate, and durable responses.\(^15\) Recently, the phase III HIMALAYA trial showed positive results for durvalumab (anti-PD-L1 antibody) as a monotherapy in combination with tremelimumab (anti-CTLA-4 antibody) for first-line treatment of advanced HCC.\(^20\) In this study, durvalumab monotherapy was non-inferior to sorafenib, and moreover the durvalumab-tremelimumab combination was superior to sorafenib (overall survival: 16.4 vs. 13.8 months).\(^20\) Based on the results of the HIMALAYA trial, durvalumab-tremelimumab combination recently obtained US Food and Drug Administration (FDA) approval as a first-line treatment for advanced HCC. Therefore, the durvalumab-tremelimumab combination offers another promising first-line treatment especially among those who cannot receive bevacizumab due to its anti-angiogenic effects.

Combinations of immunotherapy with molecularly targeted agents have mixed results. In the phase III COSMIC-312 trial, combination of atezolizumab with cabozantinib significantly improved progression-free survival compared to sorafenib (HR 0.63, 95% CI 0.44–0.91), while it did not reach statistical significance for overall survival (HR 0.90, 95% CI 0.69–1.18).\(^21\) Similarly, treatment with pembrolizumab and lenvatinib appeared to result in some improvement in overall survival and progression-free survival compared with lenvatinib monotherapy, but it did not meet statistical signifi-

**Abbreviations:**
- HCC, hepatocellular carcinoma
- PD-1, programmed cell death protein-1
- PD-L1, programmed death-ligand 1
- CTLA-4, cytotoxic T lymphocyte-associated protein 4
- HR, hazard ratio
- OS, overall survival
- SBRT, stereotactic body radiation therapy

**Keywords:** Hepatocellular carcinoma; Liver cancer; Immunotherapy

In the phase III ORIENT-32 trial, the combination of sintilimab (anti-PD-1 antibody) and IBI305 (bevacizumab bio-similar) showed significantly improved overall survival and progression-free survival compared to sorafenib in an exclusively Chinese patient cohort with high proportion of hepatitis B infection. In another phase III trial reported in 2022 (NCT03764293), the combination of camrelizumab (anti-PD-1 antibody) and apatinib was again superior to sorafenib (HR 0.62, 95% CI 0.49–0.80) and provided a median overall survival of 22.1 months, the longest overall survival observed to date in phase III trials of advanced HCC.

More recently, synergistic treatment efficacy of the combined locoregional treatment and immunotherapy have been reported and several phase 2 and 3 clinical trials are ongoing to determine the efficacy and safety of combined immunotherapy and locoregional treatment. A phase III study comparing sorafenib vs. stereotactic body radiation therapy (SBRT) followed by sorafenib showed that compared to sorafenib alone, SBRT prior to sorafenib improved overall survival (HR 0.72, 95% CI 0.52–0.99), progression-free survival (HR 0.55, 95% CI 0.40–0.75), and time to progression (HR 0.69, 95% CI 0.48–0.99) with improved quality of life.

We are in an exciting era where the landscape of systemic therapy for advanced HCC is rapidly evolving. Additional first- and second-line regimens are expected to be available as we await the readouts of ongoing phase III trials investigating different combinations of immunotherapy, molecularly targeted agents with, and without concurrent locoregional treatment. As responses to systemic therapy can be highly heterogeneous, there is an unmet need for biomarkers that would predict treatment response and enable an individualized approach to therapy.

Authors’ contribution
Ju Dong Yang devised the project and the main conceptual ideas for the snapshot; Joseph C. Ahn conducted the literature search and identified relevant studies to be included in the review; Ahn JC drafted the manuscript and the figure; Ju Dong Yang and Nguyen H. Tran revised the manuscript critically for important intellectual content; and all authors approved the final version to be published.

Conflicts of Interest
Dr. Yang provides a consulting service for Exact Sciences and Gilead. Dr. Yang’s research is funded by National Institute of Health 1K08CA259534-01A1. Dr. Tran’s research is funded by National Institute of Health 1K23MD017217-01A1.

REFERENCES


Instructions for Authors

General Information

The Clinical and Molecular Hepatology publishes original basic and clinical research on liver diseases. Manuscripts should be submitted electronically (https://mc04.manuscriptcentral.com/cmh). The journal is published in English on 1st in January, April, July, and October. Authors lacking ability with English syntax should seek the appropriate editorial assistance prior to submitting their manuscripts. These guidelines are in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals,” published by the International Committee of Medical Journal Editors at http://www.icmje.org.

The Editorial Office, the Clinical and Molecular Hepatology, Room A1210, Mapo Trapalace, 53 Mapo-daero, Mapo-gu, 04158, Seoul, Korea Tel.: 82-2-703-0051, Fax: 82-2-703-0071, E-mail: kasl@kams.or.kr

Types of Manuscripts

Contributions may be submitted as original articles, review articles, editorials and special topics. Special topics cover guidelines, meeting reports and hepatology issues elsewhere. Review articles, editorials and special topics are invited by the editorial board. However, authors who are interested in contributing reviews can submit reviews and are subjected to peer review. Letters to the editor may be subjected to peer review and undergo editing for clarity and brevity.

Ethical Conduct of the Study and the Report

All investigations involving human participants must be conducted according to the ethical guidelines of the Declaration of Helsinki, and be approved by the institutional review board. For studies involving animal experimentation, author(s) must provide assurance that all the animals received humane care according to the criteria outlined in the NIH "Guide for the Care and Use of Laboratory Animals". The author must state that the use of animals (means all mammals and birds) in the manuscript was approved by the institutional Animal Ethical Committee (AEC) in accordance to the article 14th of Korean Animal Protection Law, or equivalent, in the paper. It must be clearly stated that animal use has complied to the article 13th of Korean Animal Protection Law (The principles of animal use) and the relevant institutional polices in the manuscript. Copies of the protocol approved by institutional AEC or equivalents, must be available for review by the editor if necessary.

The corresponding author must give written assurance that neither the submitted material nor portions thereof have been published previously or are under consideration for publication elsewhere. Any material that could constitute prior or concurrent publication of similar data by any one of the authors should be submitted with the manuscript. It is assumed that the corresponding author speaks for his or her co-authors and certifies that all the listed authors meaningfully participated in the study and that they have seen and approved the final manuscript.

Authors should acknowledge any commercial affiliation or consultancy that could be 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 cultural 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.
Instructions of authors

Snapshot

Snapshot consists of a large single page figure with schematic diagrams and tables that graphically summarize current knowledge about a particular subject within the field of hepatology. A detailed figure legend which includes all relevant information can be included and may be incorporated into the main figure. The figure is accompanied by a short summary article that should not exceed a maximum of 600 words. References should not exceed a maximum of 10. The snapshot should contain a descriptive title.

1. Title page
Provide a concise title. List the full names of all authors and their institutional affiliation. In a multi-authored work involving more than a single institution, indicate individual affiliation by means of superscript Arabic numbers. Indicate a change of address in a similar fashion. List the footnotes to the title page. Provide the contact information for the corresponding author (name, address, telephone number, fax number, e-mail address and Orcid ID), and running title (Less than 50 characters). All abbreviations should be explained in this page (e.g. AFP, alpha fetoprotein; ALT, alanine aminotransferase). The Clinical and Molecular Hepatology employs a system to screen plagiarism (CrossRef). When submitting your manuscript to this journal, you accept that your manuscript may be screened for plagiarism against previously published material.

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

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

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

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

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

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

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

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

**Articles in journals**


**Literature in press**
An online article that has not yet been published in an issue can be cited by its Digital Object Identifier (DOI). The DOI will remain valid and allow an article to be tracked even after its allocation to an issue.

**Book chapters**


**Abstract or Article in a supplement**


**Websites**


10. Permissions
Direct quotations, tables or illustrations taken from copyrighted 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>
</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.

1) General Format

<table>
<thead>
<tr>
<th></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>

2) Abstract

<table>
<thead>
<tr>
<th></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>

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

<table>
<thead>
<tr>
<th></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>

4) Tables and Figures

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1] Prepare tables on individual sheets of paper, double spaced and numbered consecutively with Arabic numerals in the order of their appearance in the text.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[3] Figure legends should be typed consecutively on a separate sheet of paper.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>[4] Figures should be supplied in the JPG or TIFF format at a final resolution of 600 dpi or higher.</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Protect from Various Liver Disease with Legalon®

As the original brand of silymarin, Legalon® always be with doctors for the treatment of various liver disease.

- The original silymarin for treatment of liver disease by numerous Clinical trials since 1960's.1,2
- Originality & Worldwide

- Proven efficacy in improvement of liver function.3,4
- Treatment of Liver disease
  - NAFLD, NASH, ALD, cirrhosis

- Multi-therapeutic targets in all-stage of liver disease by various MoA.1,3
- Various MoA & All stages
  - Improvement of insulin resistance
  - Anti-oxidative stress, Anti-inflammation, Anti-fibrosis

- Good tolerance and safety with lower side effects.5,6
- Safety & Good tolerance

References:
Go to Europe in 2019

WINUF®
Enhanced ω-3 TPN

Lead the direction of Total Parenteral Nutrition

- The highest amount of Fish oil
- ω-6:ω-3=2.1:1 The ideal omega fatty acid composition ratio
- High content of Amino acids
- Provides balanced electrolytes (Zinc, α-tocopherol and other trace elements)
- Completed the first 3-phase clinical case for TPN in Korea
- Acquired the first domestic patent for the globule size stability of lipid emulsion
Effective PI-free treatment means prescribing with confidence despite unknowns\(^1\)–\(^{3,3,5}\)

EPCLUSA\(^\text{®}\) has relatively few clinically relevant DDIs and no limitations around liver disease severity\(^2\)–\(^{3,3,4}\)

### References

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, ** Ishak modified HAI(Histologic Activity Index) score

REFERENCE

M2BPGi, List Up at Liver Disease Clinical Practice Guideline

Liver fibrosis screening
Early diagnosis liver disease
Monitoring severity regularly
Single biomarker

Superior Single Biomarker Reflecting Fibrosis in All Liver Disease

Non-alcoholic Fatty Liver Disease Clinical Practice Guidelines
Updated in 2021

Non-invasive test for diagnosis liver fibrosis
Which differential tests are available for advanced fibrosis?
Advanced fibrosis can be differentiated primarily using transient elastography, FIB-4, and the NFS. When subjects are classified as intermediate risk by transient elastography, FIB-4, or the NFS, additional tests such as M2BPGi, AsAGP, ELF, SWE, or MRE can be performed.

Chronic Hepatitis B Clinical Practice Guidelines
Updated in 2022

Treatment indication
The severity of liver fibrosis can be evaluated by liver biopsy or non-invasive methods using serum markers (e.g., APRI, FIB-4 index, M2BPGi) or transient elastography (TE) using Fibroscan®.

The immune-tolerant phase is usually characterized by little or no necroinflammation without liver fibrosis, but significant fibrosis as seen using non-invasive serum fibrosis markers (e.g., APRI, FIB-4, M2BPGi) or TE (Fibroscan®) suggests that antiviral treatment can be considered.

Ref. Korean Association for the Study of the Liver Non-alcoholic Fatty Liver Disease Clinical Practice Guidelines 2021, Chronic Hepatitis B Clinical Practice Guidelines 2022

Ref. Korean Association for the Study of the Liver Non-alcoholic Fatty Liver Disease Clinical Practice Guidelines 2021, Chronic Hepatitis B Clinical Practice Guidelines 2022
**Virreal®**

Virreal® Tablets (Tenofovir disoproxil orotate) are indicated for:

1. In combination with other antiretroviral agents, for the treatment of HIV-1 infection in adult and pediatric patients 12 years of age and older.
2. For the treatment of chronic hepatitis B in adults and pediatric patients 12 years of age and older.

Dosage & Administration:

1 tablet once daily taken orally, without regard to food.

Patients with Renal Impairment:

Significantly increased drug exposures occurred in subjects with moderate to severe renal impairment. Therefore, the dosing interval of this drug should be adjusted in patients with baseline creatinine clearance below 50mL/min.

**Warnings:**

1. Lactic acidosis/severe hepatomegaly with Steatosis
2. Worsening of Hepatitis after Discontinuation of Treatment
3. New onset or Worsening of Renal Impairment
4. Coadministration with Other Products
5. Patients Coinfected with HIV-1 and HBV
6. Decreases in Bone Mineral Density
7. Fat Redistribution
8. Immune Reconstitution Syndrome
9. Early Virological Failure

**Contraindications:**

1. Hypersensitivity to this drug
2. Patients with genetic problems related to lactose

Manufactured by Dong-A ST Corp.

*Please refer to full prescribing information.*
Maviret® INDICATIONS

Maviret® may be used for 12 weeks in liver or kidney transplant recipient. A 16-week treatment duration should be considered in genotype 1, 2, 3, 4, 5, or 6 infection with compensated cirrhosis or without cirrhosis and with or without renal impairment including patients receiving dialysis.

• More than 18 hours from the usual time that MAVIRET should have been taken - advise the patient not to take the missed dose and to take the next dose at the usual time.

Hepatic Impairment: No dose adjustment of MAVIRET is required in patients with mild hepatic impairment (Child-Pugh A). MAVIRET is recommended in patients with moderate to severe hepatic impairment (Child-Pugh B or C) or those with a history of prior hepatic decompensation. Renal Impairment: No dose adjustment of MAVIRET is required in patients with mild renal impairment (creatinine clearance 50–80 mL/min) or those with prior decompensation event (i.e., prior history of ascites, variceal bleeding, encephalopathy). Rare cases of hepatic decompensation/failure were reported in patients without cirrhosis or with compensated cirrhosis (Child-Pugh A); many of these patients had evidence of portal hypertension. Events also occurred in patients taking a concomitant medication not recommended for coadministration, or in patients with confounding factors such as severe cardiovascular or neurological comorbidities. Cases typically occurred within the first 4 weeks of treatment (median 27 days). In patients with compensated cirrhosis (Child-Pugh A) or evidence of advanced liver disease such as portal hypertension, perform hepatic laboratory testing as clinically indicated, and monitor for signs and symptoms of hepatic decompensation such as the presence of jaundice, ascites, hepatic encephalopathy, and variceal hemorrhage. Discontinue MAVIRET in patients who develop evidence of hepatic decompensation.

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

Maviret® (glecaprevir/pibrentasvir fixed-dose combination tablet) is a direct-acting antiviral agent (DAA) for the treatment of adult and adolescent patients (12 years and older) with chronic hepatitis C virus (HCV) infection.

Table 1. Recommended Duration for Treatment-Naive Patients

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Recommended Treatment Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5, or 6</td>
<td>8 weeks</td>
</tr>
</tbody>
</table>

Table 2. Recommended Duration for Treatment-Experienced Patients

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>Recommended Treatment Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>An NS3/4A protease inhibitor 8 weeks</td>
</tr>
<tr>
<td>2</td>
<td>An NS3/4A protease inhibitor 12 weeks</td>
</tr>
<tr>
<td>3</td>
<td>An NS3/4A protease inhibitor 16 weeks</td>
</tr>
</tbody>
</table>

[Image]
Yellow, round, film-coated tablets, debossed with “GSI” on one side of the tablet and “25” on the other side

**INDICATION**

This drug is indicated for the treatment of chronic hepatitis B in adults.

**DOSEAGE AND ADMINISTRATION**

The recommended dosage of this drug is one tablet taken orally once daily, with or without food.

**PRECAUTIONS IN USE**

1. **Warnings**
   - This drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. For patients who are breastfeeding, this drug should not be used.
   - Lactic acidosis/severe hepatomegaly with steatosis and severe hyperamylasemia have been reported with the use of nucleotide analogues; patients using this drug should be monitored for these adverse effects.
   - Vemlidy is contraindicated in patients with a history of severe hypertriglyceridemia.

2. **Contraindications**
   - This drug is contraindicated in patients with a history of severe hypertriglyceridemia.

3. **Precautions**
   - This drug should be used with caution in patients with a history of severe hypertriglyceridemia.

**INTERACTIONS**

1. **Pharmacokinetic**
   - This drug is a substrate of P-glycoprotein (P-gp) and BCRP. Drugs that strongly inhibit P-gp and BCRP may increase the risk of adverse reactions.
   - This drug should be used with caution in patients with a history of severe hypertriglyceridemia.

**ADVERSE REACTIONS**

1. **Infections**
   - This drug may increase the risk of infections, including bacterial, viral, and fungal infections.

2. **Liver Function Tests**
   - This drug may increase the risk of liver function tests, including ALT, AST, ALP, and total bilirubin.

**EFFECTS ON LABORATORY TESTS**

1. **Renal Function Tests**
   - This drug may increase the risk of renal function tests, including serum creatinine and blood urea nitrogen.

**PREGNANCY**

1. **Pregnancy**
   - This drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.
   - It is not known whether this drug and its metabolites are present in human breast milk, affect human milk production, or have potential adverse effects on the breastfed infant.

**NURSING MOTHERS**

1. **Lactation**
   - This drug should not be used by breastfeeding women.

**GERIATRIC USE**

1. **Use in the Elderly**
   - This drug should be used with caution in elderly patients.

**DOSEAGE AND ADMINISTRATION**

- The recommended dosage of this drug is one tablet once daily, taken orally, with or without food.
- The drug should be taken at the same time each day.
- Patients should take the tablet whole and swallow it without breaking, crushing, or chewing it.

**STORAGE**

- Store in a tight container at room temperature (1-30°C).
- Protect from light.

**REFERENCES**

1. [Reference 1](#)
2. [Reference 2](#)
3. [Reference 3](#)
4. [Reference 4](#)
5. [Reference 5](#)
6. [Reference 6](#)
Gilead Liver Commitment
Exploring for Complete Understanding of Liver Disease
RECOGNIZE & KILL CANCER CELLS

Paradigm Shift in Cancer Treatment

Recognize & Kill the cancer cells

Immuncell-LC
Anticancer cellular Immunotherapeutics

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, once every two weeks, 4 times, once four weeks, 4 times, once every eight weeks 16 times in total.
The new wave of GERD Treatment, P-CAB

Fexuprazan hydrochloride

Full and fast onset of effect with the first dose

Rapid and superior heartburn symptom relief

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.

Fexu

CMH v29 n1 14 | 4월 03일 11

The Product is administered to adults as follows. Treatment of erosive esophagitis (EE) - 40 mg is administered orally once a day for 4 weeks. In case of patients with untreated esophagitis or symptoms persisting, the administration given for another 4 weeks. The Product can be administered with or without meals.

Contraindications
1) Patients who have a history of hypersensitivity to the Product or its components
2) Patients taking a drug containing atazanavir, nelfinavir, or rilpivirine (refer to '5. Interactions')
3) Pregnant and lactating women (refer to '6. Administration to Pregnant and Lactating Women')
4) Patients who have congenital conditions for lactose such as galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption, as this medicine contains lactose.

The following patients should be administered with care.
1) Patients with hepatic impairment (no experience of use)
2) Patients with renal impairment (no experience of use)
3) Elderly (refer to '8. Geriatric Use')
4) Patients who have a history of hypersensitivity or allergy to Yellow 4 Tartrazine

Storage
Store at temperatures not exceeding 30ºC in an airtight container.

Shelf-life
36 Months

Availability
28T/Box (7T/PTP*4), 56T/Box (7T/PTP*8), 28T/Btl, 100T/Btl, 300T/Btl

Manufactured by
Daewoong Pharmaceutical Co., Ltd./ 1, Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Republic of Korea

This medicine has passed strict quality control. If the use-by date or expiration date has passed, it is no longer safe and effective. In case of damage, spoilage, contamination, or other issues, contact the pharmacy or drug distributor where it was purchased.

For detailed and up-to-date approval information, please refer to the Ministry of Food and Drug Safety's integrated drug information system (https://nedrug.mfds.go.kr) or the product label.
FibroScan®
by echosens

The non-invasive gold standard solution for comprehensive management of liver health

**CAP 신의료기술 고시**
보건복지부 고시 제2021-163호, 2021.6.7

**New! Fibroscan 630 Expert**
Spleen Stiffness Measurement (SSM by VCTE) Surrogate marker of PH

**Scores (Agile 3+ & 4) by Echosens**
LSM과 혈액 바이오 마커(AST, ALT, Plt)
결합 및 계산하여 NAFLD 환자의 F3/F4를 식별합니다

**Surrogate marker of liver fibrosis**
- Measurement of liver stiffness (expressed in kPa)
- Relevant in all Chronic Liver Diseases (CLD)

**Surrogate marker of liver steatosis**
- Measurement of ultrasound attenuation (expressed in dB/m)
- Relevant in Fatty Liver Diseases: AFLD, NAFLD, NASH

**Surrogate marker of portal hypertension (PH)**
- Shear wave frequency of 100Hz
- Relevant in the diagnosis of large esophageal varices
- Relevant for the risk stratification of cirrhotic patients

에크미디칼(주) TEL. 02 585 1291
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, Adenosine HCl 2.5mg, Pyridoxine HCl 2.5mg, Riboflavin 0.5mg, Cyanocobalamin 0.125mg, Biphenyl dimethyl dicarboxylate 25mg
- Indication: I) 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. Doseage 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 antihistamn 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 nausa, gastric discomfortness can be represented. 4) Rarely itching or redness can be occur, in this case, discontinue administration and follow physicians instruction.
- Insurance Code: 6931000080 4) Packaging Unit: 100, 100 caps. (bottle) 100 caps. (PTP)
- Storage: Tight closed container, room temperature (1~30°C) in dry place. Expiry - 6 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
Lividi is good choice for patients with elevated ALT

- Decrease elevated ALT significantly. -CCL₄ Detoxication
- Stabilize cell membranes
- Improve cholestatic index

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


SOR-1-2012-01
I had the will to start a business from scratch. But I still need help to lose weight and keep it off.

SARAH, Age 43, BMI 37 (Patient portrayal)

For people with obesity, losing weight and keeping it off is more than a matter of willpower. Changes in appetite-regulating hormones after weight loss drive weight regain, undermining their efforts. Saxenda® is 97% similar to natural GLP-1, a hormone that works in the brain to decrease appetite and thereby reduce food intake, leading to significant and sustained weight loss.

Obesity is a chronic disease, and most patients want your help. Ask your patients about their weight loss attempts, and tell them how adding Saxenda® to diet and exercise can help them lose weight and keep it off.

Your patients with obesity have the will. You can offer them the way.
Remarkable Response

The ORR was more than three times higher with lenvatinib versus control group. Based on the masked IIR according to mRECIST, about 41% of patients* showed ≥ 30% decrease in tumor size.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 abstract of the article (Kudo M, et al. 2018)

**Table**

<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>

mRECIST: modified Response Evaluation Criteria in Solid Tumors; IIR: Independent Imaging review; ORR: Objective Response Rate; CI: Confidence Interval; OR: odds ratio; 95% CI: 95% confidence interval


**Legend**

Lenvinib: Lenvratinib; Sorafenib: Sorafenib; mRECIST: modified Response Evaluation Criteria in Solid Tumors; IIR: Independent Imaging review; ORR: Objective Response Rate; CI: Confidence Interval; OR: odds ratio; 95% CI: 95% confidence interval


**References**


**European Union Registration**

Lenvatinib is approved for the treatment of patients with recurrent or progressive differentiated thyroid carcinoma, with mRECIST-defined Progression-Defined Objective Response Rate (pDOR) of ≥ 40% in pivotal phase II and phase III studies.

**Canadian Registration**

Lenvatinib is approved for the treatment of patients with advanced or recurrent differentiated thyroid carcinoma, with mRECIST-defined Progression-Defined Objective Response Rate (pDOR) of ≥ 40% in pivotal phase II and phase III studies.

**References**


**European Union Registration**

Lenvatinib is approved for the treatment of patients with recurrent or progressive differentiated thyroid carcinoma, with mRECIST-defined Progression-Defined Objective Response Rate (pDOR) of ≥ 40% in pivotal phase II and phase III studies.

**Canadian Registration**

Lenvatinib is approved for the treatment of patients with advanced or recurrent differentiated thyroid carcinoma, with mRECIST-defined Progression-Defined Objective Response Rate (pDOR) of ≥ 40% in pivotal phase II and phase III studies.

**References**

Ramnos® not only strengthens intestinal health and immunity, but also improves atopic symptoms.
Damaged Livers Can Be Recovered

The Only Korean Medicine Proven to Reduce the Level of MDA, a Biomarker of Oxidative Stress, Through Phase IV Clinical Trials
Significantly Reduced the Level of MDA in Alcoholic Hepatitis, Nonalcoholic Steatohepatitis and Viral Hepatitis Patients

Safe Medicine Proven to Improve Quality of Life for Patients
Patients’ Improved Quality of Life Verified Through Chronic Liver Disease Questionnaire (CLDQ)

Antioxidative Effect Reduces Fat in the Liver
Proven to Reduce MDA Level

Proven Efficacy
Quickly Reduces and Helps You Maintain Optimal Level of Alanine Transaminase (ALT)
Contains Garlic Oil Which is Known to Have Strong Antioxidative and Anti-Inflammatory Effects

Ingredients]
Chronics hepatitis with continuously elevated ALT level
Directions] Take 1 or 2 capsules each time, 3 times a day, after meals

Diagnostic Code
B15-19 Viral hepatitis K70.0 Alcoholic fatty liver K71.0 Toxic liver disease K73.0 Chronic persistent hepatitis, NEC K74.0 Hepatic fibrosis K75.8 Other specified inflammatory liver disease, Nonalcoholic steatohepatitis K77.0 Liver disorders in disease classified elsewhere
SK Albumin Inj 5%/20%

Human serum albumin

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

Summary of Prescribing Information

**PRODUCT NAME** SK Albumin 5%/20% Inj
**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** Hypoalbuminemia caused by albumin loss (burn, nephrotic syndrome, etc.) and dysfunction of albumin synthesis (liver cirrhosis, etc.), hemorhagic shock
**DOSEAGE 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. 125-250 mL, equivalent to human serum albumin 25-50 g should be administered by intravenous drip infusion or by slow direct intravenous injection. The recommended infusion rate is 3-4 mL/min. It may be diluted with 5 % glucose when necessary. The dosage may be adjusted according to body weight, age and symptoms. **CONTRAINICATION** Patients with a history of hypersensitivity reactions to this drug and its components

**MANUFACTURER** SK Plasma Co., Ltd. (36918) 157 Sanoeopdanipil, Pungsan-eup, Andong-si, Gyeongsangbuk-do, Republic of Korea

**MA HOLDER** SK Plasma Co., Ltd. (118494) 310 Pungsan-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea

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

STRIKE FIRST

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

Proven.
Personalized.
Precise.
Reformulation of MG OS by utilizing NanoCrystal® Technology.
50 times smaller particles increased surface area
rapid dissolution & increased absorption.
Improved bioavailability

I. Originality
- licensed the Megace® name from Bristol-Myers Squibb Company
- High Quality

II. Improved Bioavailability!
*** Food effect differences between a nanocrystal dispersion of megestrol acetate 625 mg/5 mL and a micronized formulation of megestrol acetate oral suspension(MGOS) 800 mg/20 mL. ***

III. Improved Efficacy!
- Weight gain occurred more rapidly at each time point
- Patients in the nanocrystal dispersion arm gained an average of 10% of the baseline weight over 12 weeks (Vs 6% weight gain in MG OS arm )

IV. Improved Convenience!

<table>
<thead>
<tr>
<th></th>
<th>Megace F-OS</th>
<th>Megace OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended dose</td>
<td>1 teaspoon</td>
<td>4 teaspoon</td>
</tr>
<tr>
<td>Concentration</td>
<td>625 mg/5mL</td>
<td>800 mg/20mL</td>
</tr>
<tr>
<td>Viscosity</td>
<td>10 cP</td>
<td>163 cP</td>
</tr>
</tbody>
</table>

* cP: centipoise, a measure of viscosity, with higher numbers indicating greater viscosity.

International Journal of Nanomedicine 2009; 4:185-192

- 1/4 the total volume per dose
- 94% less viscous — easier to take