Together, elimination is happening here.

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HARVONI - THE ONLY "PAN-FIBROTIC" PI FREE "STR".
Gilead Liver Commitment
Exploring for Complete Understanding of Liver Disease
VEMLIDY® is indicated for the treatment of chronic hepatitis B virus (HBV) infection in adults.

TARGETED DELIVERY OF TENOFOVIR, FOR TODAY AND TOMORROW

Powerful antiviral efficacy with improved rates of ALT normalization
- Shown comparable viral suppression vs. VIREAD
- Deliver higher rates of ALT normalization vs. VIREAD
- 0% Detectable-resistance at 96 week

Improved safety profile in renal and bone parameters
- Less impact on renal and bone safety parameters as compared to VIREAD

Recommended 1st line treatment option in KASL, EASL and AASLD guideline

Provides targeted delivery to the liver reducing systemic exposure with 89% lower plasma concentration of tenofovir compared to VIREAD

References
8 WEEKS: THE SHORTER ROUTE TO HCV CURE†

Treatment-naive without cirrhosis or with compensated cirrhosis

**Single 8-week Duration**
for treatment-naive patients† (GT 1-6)*

**98% High Cure Rates (98%)**
all genotypes, treatment-naive

**Once-daily Dosage**
3 tablets, 4 times a day

The type and severity of adverse reactions in subjects with compensated cirrhosis were comparable overall to those seen without cirrhosis.

DG=compensated cirrhosis; ITT=intent-to-treat.

MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C).† MAVIRET is indicated for the treatment of chronic hepatitis C virus (HCV) infection in adults and adolescents aged 12 to <18 years.

*Refers to GT 1-6 including treatment-naive patients with GTs and compensated cirrhosis, 2) decompensated cirrhotic patients and 3) Liver or kidney transplant recipients. MAVIRET is not indicated in decompensated cirrhosis. The recommended duration of MAVIRET is 12 weeks, for treatment-naive patients with GTs and compensated cirrhosis and 2) Liver or kidney transplant recipients, with or without cirrhosis. The maker for use means swallowed whole with food and not chewed, crunched, or broken.


Maviret® (darunavir/cobicistat/emtricitabine/tenofovir alafenamide) tablets in blister packs. Each blister contains ten tablets, one of which may be uncoated. MAVIRET tablets should be swallowed whole and not chewed, crunched, or broken. MAVIRET is contraindicated in patients with moderate or severe hepatic impairment (Child-Pugh B or C).† MAVIRET is indicated for the treatment of chronic hepatitis C virus (HCV) infection in adults and adolescents aged 12 to <18 years.

**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></td>
<td>Naive</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, or 6</td>
<td>8 weeks</td>
</tr>
<tr>
<td>1</td>
<td>12 weeks</td>
</tr>
<tr>
<td>2</td>
<td>12 weeks</td>
</tr>
<tr>
<td>3</td>
<td>12 weeks</td>
</tr>
<tr>
<td>4</td>
<td>12 weeks</td>
</tr>
<tr>
<td>5</td>
<td>12 weeks</td>
</tr>
<tr>
<td>6</td>
<td>12 weeks</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>Patients Previously Treated With A Regimen Containing</th>
<th>Recommended Treatment Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dargavir/ribavirin/sofosbuvir</td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>Dargavir/ribavirin/sofosbuvir</td>
<td>12 weeks</td>
</tr>
<tr>
<td></td>
<td>Dargavir/ribavirin/sofosbuvir</td>
<td>12 weeks</td>
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<td>Dargavir/ribavirin/sofosbuvir</td>
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<tr>
<td></td>
<td>Dargavir/ribavirin/sofosbuvir</td>
<td>12 weeks</td>
</tr>
</tbody>
</table>

**Simple Hepatitis**

Dose adjustment of MAVIRET is required in patients with mild hepatic impairment (Child-Pugh A; MAVIRET). MAVIRET is contraindicated in patients with moderate to severe hepatic impairment (Child-Pugh B or C) or those with any history of hepatic decompensation. No dose adjustment of MAVIRET is required in patients with mild or severe renal impairment.

**CAUTIONS**

MAVIRET may be used for 12 weeks in liver or kidney transplant recipients. A shorter treatment duration should be considered in transplant patients with previous infection who are NASH, multigenerational, or treatment-naive or with prior steatohepatitis or decompensation.

**ADVERSE REACTIONS**

MAVIRET is contraindicated in patients with moderate to severe hepatic impairment (Child-Pugh B or C) or those with any history of hepatic decompensation. MAVIRET is contraindicated in patients with moderate to severe renal impairment. MAVIRET is contraindicated in patients with moderate to severe renal impairment. MAVIRET is contraindicated in patients with moderate to severe renal impairment. MAVIRET is contraindicated in patients with moderate to severe renal impairment. MAVIRET is contraindicated in patients with moderate to severe renal impairment. MAVIRET is contraindicated in patients with moderate to severe renal impairment.
Hepsera Regained control, Sustained control

In LMV-resistant patients, combined ADV-LMV therapy attenuated the risk of genotypic resistance to ADV, preventing virologic and clinical breakthrough during a 3-year period.

Hepsera. Extending your power to fight hepatitis B.

Hepsera. Taking your patients further.

References


Safety Information

1. Severe Hepatitis: If liver function must not be substituted. 2. Treatment interruption is necessary to avert the risk of hepatic deterioration. 3. Treatment interruption for hepatitis B patients should be carried out with caution. 4. Treatment interruption for hepatitis C patients should be慎行。5. Treatment interruption for hepatitis C patients should be carried out with caution. 6. Treatment interruption for hepatitis C patients should be carried out with caution.

Abbreviated Prescribing Information Version 04

Hepsera (lamivudine disodium trihydrate) is indicated for the treatment of chronic B hepatitis patients with evidence of active replication and other evidence of active disease in serum Hepatitis B virus (HBV) DNA levels greater than 10^5 copies/mL.

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Hepsera is contraindicated in patients with active hepatitis B or other severe hepatic disease, or with uncontrolled HIV infection.

Hepsera is not a virostatic but has a strong anti-HBV effect, with the ability to reduce viral load and the risk of disease progression. It is important to use Hepsera in combination with other antiviral agents, such as interferon or other antiviral agents, to achieve the best possible virological response.

Hepsera is well tolerated, with a low incidence of adverse events. The most common adverse events reported in clinical trials were nausea, vomiting, diarrhea, abdominal pain, and headache. Hepsera is not associated with the development of viral resistance. Hepsera is not recommended for use in patients with inactive hepatitis B or for patients with chronic hepatitis B who have already achieved virological response to previous antiviral therapy.
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GC Pharma
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**VCTE™**
Vibration Controlled Transient Elastography
- Assess liver stiffness to quantify fibrosis, cirrhosis and other parameters
- Provide reproducible and operator independent examination\(^1\)
- Explore a large volume (100 times larger than the biopsy)

**CAP™ (option)**
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- CAP is a measure of the ultrasound attenuation to quantify steatosis in the liver
- Liver Stiffness Measurement (fibrosis) and CAP (stéatosis) are simultaneously measured in the same liver volume
- CAP is measured at 3.5 MHz and is expressed in decibel per meter (dB/m)
- CAP can be measured with M and XL probes

FibroScan® measures liver stiffness that is directly related to liver conditions such as fibrosis, inflammation\(^2\).
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SOFT LIVER = NORMAL    STIFF LIVER = FIBROSIS

---

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Evidenced by numerous clinical results

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- 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 25mg, Riboflavin 0.5mg, Cyanocobalamin 0.125mg, Bipheryl dimethyl dicarbosylate 25mg
- Indication: 1) General therapeutics for the following hepatic disease - Acute, Subacute and Chronic Hepatitis, Hepatic cirrhosis, Fatty liver, Drug or chemical induced hepatitis 2) Acute, chronic hepatitis involving high transaminase value
- Dosage & Administration: Usually, each time 2 capsules, 2–3 times a day as adult dosage. Dosage unit can be changeable depending on symptom or age of patient.
- Special caution: 1) Severe state of chronic hepatitis 2) Severe state of hepatic cirrhosis
- General caution: 1) Rarely skin rash can be represented, in this case general antihistamin therapy will be required. 2) In severe case, sometimes intermittent jaundice can be occur in this case, discontinue administration for awhile and other adjuvant therapy for jaundice shall be required. 3) Rarely nausea, gastric discomfort can be represented. 4) Rarely itching or reddiness can be occur, in this case, discontinue administration and follow physician's instruction.
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- Packing Unit: 100, 300 caps. (bottle)/100 caps. (PFP)
- Storage: Tight closed container, room temperature (1 ~ 30°C) in dry place. Expiry - 60 months from Manufacturing date.

Diagnostic Codes
- B15-19: Viral hepatitis K70.0: Alcoholic fatty liver K71.0: Toxic liver disease K73.0: Chronic persistent hepatitis, NEC K74.0: Hepatic fibrosis K75.8: Other specified inflammatory liver disease, Nonalcoholic steatohepatitis K77.0: Liver disorders in disease classified elsewhere
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The first developed nucleotide analogue in Korea. Efficacy, safety and beneficial effects of L-carnitine PLUS!

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• Besivo has antiviral efficacy comparable to that of TDF after 48 weeks of treatment, with durable effects for 96 weeks.

Tolerance of Besivo®
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Histological effect of Besivo®
• Besivo showed a significantly higher proportion of patients with improved histological scores than TDF.

* Ishak modified HAI (hepatic activity index) scoring system
TDF: Tenofovir disoproxil fumarate

REFERENCES
2. Besivo Phase III Clinical Trial. Protocol No. ID_BVCL011 Clinical Study Report
Aims and Scope

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

Clin Mol Hepatol publishes original papers, meta-analysis, letter to editor, case reports, reviews, guidelines, editorials, and liver image and pathology on all aspects of the field of hepatology.

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Special Topic

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INTRODUCTION

Coronavirus disease-19 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is spreading rapidly all over the world, including Korea, which was firstly reported from Wuhan, China. Even in this pandemonium, the treatment of patients with chronic liver disease, hepatocellular carcinoma (HCC), and management associated with liver transplantation should be elegantly continued. Precautions to be undertaken with knowledge of COVID-19 are summarized in this document.

DETAILS AND RECOMMENDATIONS

Effect of SARS-CoV-2 virus on the liver

1) SARS-CoV-2 binds to cells through angiotensin-converting enzyme 2 (ACE2). ACE2 is highly expressed in hepatocytes and bile duct epithelial cells. Thus, hepatocytes and bile duct epithelial cells can become target cells for viral infection. However, there is no evidence of viral inclusion within liver tissue.

2) Liver function test (LFT) abnormalities. (1) Incidence: 14–53%. Rare cases of acute liver damage have been reported in COVID-19 patients. (2) Presented as: elevated serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase, and bilirubin. LFT abnormalities can be caused by immune-cytopathic damage following direct cytotoxicity and inflammatory response of SARS-CoV-2. It is difficult to distinguish whether liver dysfunction is due to SARS-CoV-2 itself, its complications, or side effects caused by therapeutic drugs. (3) SARS-CoV-2 can influence the disease course of chronic liver disease due to its direct toxicity to the liver, reactivation of preexisting chronic hepatitis virus, and liver damage from medications targeting the SARS-CoV-2. Experimental drugs such as remdesivir,
chloroquine, hydroxychloroquine, tocilizumab, and statins used as therapeutic agents for COVID-19, along with antibiotics and antifungal agents used as treatments for accompanying bacterial and fungal pneumonia may induce liver toxicity. In a Chinese randomized controlled trial of remdesivir, serum bilirubin was elevated in approximately 10% of subjects in the remdesivir group, which was not significantly higher than that (9%) in the placebo group. AST elevation was also reported in 5% of subjects in the remdesivir group, which was not significantly different from the placebo group (12%). In another randomized controlled trial of remdesivir vs. placebo, the elevation of AST and ALT was not significantly more frequent in the remdesivir group than that in the placebo group. (4) LFT abnormalities are more common in patients with severe COVID-19 than in patients with mild cases. (5) Hypoalbuminemia at hospitalization reflects the severity of COVID-19. (6) Hepatic dysfunction is generally transient and does not require special treatment.

3) Data on liver histology of patients with COVID-19 are limited and its characteristics are mainly nonspecific.

[Considerations]
1. If a COVID-19 patient has an abnormal liver function, serological tests including hepatitis B surface antigens and anti-hepatitis C virus antibody are necessary as in the case of LFT abnormality under normal circumstances.
2. If COVID-19 is positive in patients with autoimmune hepatitis and those who have undergone liver transplantation, deterioration in LFT should not be presumed as a disease flare or an acute rejection without a biopsy confirmation.
3. To avoid infections while transporting a COVID-19 patient, cancel or postpone diagnostic tests including ultrasound, computed tomography, magnetic resonance imaging unless bile duct obstruction, cholangitis, and acute venous thrombosis are suspected.
4. Abnormality in liver function tests is not a contraindication in the use of experimental or off-label treatments for COVID-19 (remdesivir, chloroquine, hydroxychloroquine, tocilizumab, statin, etc.). However, regular monitoring of liver function tests is necessary in these patients regardless of baseline liver function test results.
5. When a patient’s liver function continues to deteriorate, other causes should be considered.

A. Consider COVID-19 complications such as myositis (especially when AST exceeds ALT), ischemia, cytokine release syndrome, and drug-induced liver injury.

Management of outpatients with stable liver disease

1) Interindividual transmission of SARS-CoV-2 may occur even from asymptomatic patients to both immunocompromised and immunocompetent groups.
2) In children, severe or fatal COVID-19 is uncommon and increases in ALT and AST are usually mild. However, transmission of the virus is still possible.
3) About 0.6% of COVID-19 patients had chronic liver disease according to report from Centers for Disease Control and Prevention (CDC) of the USA. Approximately 2–11% of Chinese COVID-19 patients accompanied chronic liver disease.
4) Approximately 1% of COVID-19 patients were reported to have a history of cancer. They had a higher risk of intensive care unit care or death. The incidence of COVID-19 or the risk of death associated with SARS-CoV-2 in HCC patients are unknown.
5) The median tumor doubling time of 4–6 months in HCC could be the rationale for a brief delay of radiological surveillance.

[Considerations]
1. Postponing an appointment or considering a non-visit, non-face-to-face interview is necessary in the management of patients with stable liver disease.
2. Health care provider should minimize the risk of infection during patients’ and accompanying guardians’ hospital stay.
3. Check COVID-19–related symptoms (fever, coughing, shortness of breathing, sore throat, diarrhea, etc.) or history of travel to epidemic areas prior to appointment. Reschedule an appointment if necessary.
4. All patients who visit the hospital should be screened at the entrance for respiratory symptoms and measured body temperatures. Patients with symptoms or febrile diseases should be treated in separate screening clinics for COVID-19. They should not be permitted to hepatology clinic or liver transplant clinics.
5. Surveillance and monitoring for HCC are recommended to maintain pre-scheduled visits, but short-term delay is possible.
considering the lack of capacity in medical services amid COVID-19 pandemic according to each institution and patients’ personal circumstances. (It is necessary to discuss benefits and risks caused by a delay in cancer monitoring with the patient and a documentation of the explanation is recommended.)

6. Treatment of HCCs should be continued regardless of the COVID-19 pandemic.

Liver transplantation and post-transplantation care

There is no evidence that the severity of COVID-19 infection increases in patients after liver transplantation. (1) According to data reported up to date, pulmonary injury is increased due to innate immune response to SARS-CoV-2 and immunosuppression might have a protective role.\(^5,10,21\) (2) In cases of severe acute respiratory syndrome (SARS, 2003–2004) and Middle East respiratory syndrome (MERS; from 2012), immunosuppression after liver transplantation did not increase the risk of mortality.\(^20\) (3) According to a case report, a patient who was tested positive for COVID-19 immediately after liver transplantation became negative for COVID-19 after treatment but returned to positive after increasing the dosage of immunosuppressants.\(^22\)

Management of patients with decompensated cirrhosis or waiting for a liver transplant

1) The effects of SARS-CoV-2 infection on patients with chronic liver disease, decompensated cirrhosis, and awaiting liver transplantation are not clearly understood.

2) Based on previous experience with SARS-CoV, patients with chronic liver disease (chronic hepatitis B and C) are expected to be more vulnerable to COVID-19 infection,\(^7\) but some studies have reported that patients with chronic liver disease do not have worse prognosis.\(^18\) Therefore, it is too early to make a conclusion with the evidence that we have so far.

3) A nationwide study in the USA showed that cirrhosis hospitalizations declined during the COVID-19 pandemic and model for end-stage liver disease (MELD) score at admission was higher in the late-COVID era compared to the previous period.\(^19\) This finding suggests that the COVID-19 pandemic delayed admission of cirrhotic patients possibly due to limited medical resources. Such indirect secondary effect of COVID-19 on cirrhotic patients needs to be considered.

[Considerations]

1. SARS-CoV-2 positive patients are limited as candidates for organ donation.
   A. Deceased donors: All deceased donors should be tested for SARS-CoV-2 before donation. They should be confirmed as negative for SARS-CoV-2 before performing an organ harvesting surgery.\(^23\) A person with the possibility of having been in contact with a patient confirmed or suspected for COVID-19 within 14 days should not be considered as a donor. Death by unknown respiratory failure should also be considered as an exclusion criterion.
   B. Living organ donors and transplant recipients: Transplantation surgery for living donors and recipients who have visited an epidemic area or who are concerned about an epidemiological relevance should be deferred for 14 days of observation. For all donors and recipients, SARS-CoV-2 test result must be confirmed as negative before an operation.\(^23\)

2. Medical resources (intensive care unit, ventilator, blood products, etc.) must be secured prior to transplantation.

3. Reduction of immunosuppressant or discontinuation of mycophenolate is not recommended for asymptomatic liver transplant patients unless they are COVID-19 positive.

4. The possibility of prolonged viral shedding caused by the use of immunosuppressants in transplanted patients demands attention to post-transplantation care. Examinations should be conducted in accordance with guidelines for each medical institution in case of suspected COVID-19.

[Considerations]

1. Patients with HCC or severe liver disease with high MELD scores can visit medical institutions for pre-transplant evaluation while avoiding unnecessary blood or imaging tests.
2. Educate patients not to travel during the COVID-19 pandemic.
3. Pre-transplant education should be replaced with video materials if possible. Multiple patients should not be gathered for education in a limited space. Pre-transplant counseling should also be replaced by telephone counseling.
4. Admission to a transplantation ward is strongly restricted for patients with COVID-19 symptoms.

http://www.e-cmh.org

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Treatment of HCC patients

1) As the COVID-19 pandemic state is prolonged, shortage of medical devices and health care providers may occur.
2) A multidisciplinary team approach is recommended to plan treatment method and appropriate time for HCC patients while considering the risk of in-hospital infection.
3) Treatment of HCC can be undertaken at the public relief hospitals.
4) HCC patients should minimize visits to hospitals in order to decrease the risk of infection. Invasive treatments should be considered for patients with low possibility of decompensation and minimal risk for developing severe COVID-19.

[Considerations]
1. There is no need to delay necessary treatment unless there is a shortage of medical devices or health care providers.
2. For patients with a high risk of developing severe COVID-19, treatment should be decided after evaluating risks and benefits.
3. Treatment response evaluation can be extended temporarily according to COVID-19 pandemic status.
4. When a patient is on a clinical trial, rearrangement of hospital visits, treatment schedule and treatment site can be considered.
5. If it is difficult to apply the first recommended treatment modality, optimal alternative therapy or intensive imaging examinations should be performed.
6. Liver transplantation can be delayed in order to protect the donor and the recipient. In such a case, the patient should be fully informed about the possibility of cancer progression and deterioration of liver function.
7. While waiting for liver transplantation, bridging therapies could be considered.
8. Visiting schedules can be rearranged in order to minimize hospital visits of patients who are on systemic therapy. Remote medical support can be considered if possible.
9. For patients receiving treatment for HCC, the risk of infection upon hospital visit should be considered.
10. If treatment continuation is not feasible, intensive imaging examinations or conservative management should be done.
11. For HCC patients who develop COVID-19, locoregional therapies should be delayed. Consideration to temporarily withdraw immune-checkpoint inhibitors should be undertaken as there is a higher risk of developing serious COVID-19 in patients receiving immunosuppressive therapy. In mild cases of COVID-19, the continuation of kinase inhibitors should be in the discretion of the clinician’s judgement with each case.

The use of immunosuppressants

1) Effects of immunosuppression on the development of COVID-19 have not been established yet.
2) Rapid exacerbation of respiratory symptoms has been reported to be systemic and respiratory inflammatory reactions related to increased levels of serum interleukin (IL)-6, IL-8, and tumor necrosis factor-α.
3) Potential benefits of corticosteroids in preventing the progression of symptoms in mild COVID-19 patients have not been well understood yet. However, the use of steroids in SARS and MERS patients was not advantageous in a meta-analysis.
4) In patients with autoimmune liver disease or patients with acute rejection after liver transplantation, rapid reduction or discontinuation of immunosuppressive agents can exacerbate the course of the disease.

[Considerations]
1. Use of immunosuppressants in COVID-19–negative patients
   A. Adjustment of the dose of immunosuppressants in advance due to the COVID-19 pandemic is not necessary.
2. Use of immunosuppressants in COVID-19–positive patients
   A. Steroids or immunosuppressants can be used when potential benefits are greater than the risk.
   B. Consider minimizing the dosage of high-dose steroids, but maintain a sufficient dose to avoid adrenal insufficiency or aggravation of underlying liver disease that has been controlled by steroids.
   C. In cases of pneumonia aggravation, lymphopenia and persisting fever, the daily dose of azathioprine or mycophenolate can be reduced or discontinuation of the drug can be considered according to the discretion of the clinician’s judgement.
   D. In cases of pneumonia aggravation, lymphopenia, and persisting fever, the daily dose of calcineurin inhibitors may be reduced. However, such inhibitors should not be discontinued since calcineurin inhibitors as cornerstones
of immunosuppression cannot be replaced by other immunosuppressants, especially in post-transplant patients.

3. Modulating immunosuppressants after liver transplantation in COVID-19 patients
   A. >6 months from liver transplant: Consider reducing or stopping antimetabolites. Consider reducing the level of calcineurin inhibitor.
   B. <6 months from liver transplant: Consider reducing or stopping antimetabolites. Maintain calcineurin inhibitor.
   C. Early post-transplant period: Consider maintaining immunosuppressants.

Inpatient care and inpatient/outpatient procedures

1) The possibility of fecal-to-oral transmission of SARS-CoV-2 has been suggested\(1,2,7,28\) and the virus has been identified in saliva.\(^{29}\)
2) The stability of SARS-CoV-2 in aerosols has been confirmed,\(^{30}\) suggesting the possibility of virus transmission via aerosols.\(^{31}\)

[Considerations]

1. Before admission, check travel history to prevalent areas, fever, and respiratory symptoms. Delaying hospitalization should be considered for symptomatic patients. If the emergency patient with pneumonia of unknown origin is recommended to be admitted to an isolated unit, COVID-19 infection should be tested.
2. All visitors should be checked for their travel history and symptoms such as fever, cough, and sore throat when they visit the hospital. Their access should be recorded and visits other than one guardian are strictly restricted.
3. In patients with liver disease diagnosed with COVID-19, non-emergency procedures such as elective endoscopy, liver biopsy, and etc. should be postponed as much as possible. However, urgent or emergent procedures (e.g., liver biopsy to confirm graft rejection in transplant patients, therapeutic paracentesis, transjugular intrahepatic portosystemic shunt, endoscopy for treatment of variceal bleeding, and pancreatico-biliary procedures) should be performed promptly.
4. During all medical procedures, healthcare workers should comply with standard precautionary guidelines. They should wear goggles or face shields and gloves when an exposure to secretions is expected and apply KF94 or N95 masks when aerosol spread of the virus is concerned.\(^{32,33}\)

5. After all procedures, follow appropriate disinfection guidelines for each procedure. Follow the Korean CDC instructions after performing procedures for confirmed or suspected COVID-19 patients.\(^{33,34}\)

Medication management and potential drug-drug interactions in COVID-19 patients

1) US Food and Drug Administration granted emergency use authorization for investigational antiviral remdesivir to treat COVID-19.
2) Remdesivir, a nucleotide analogue, is currently undergoing multiple randomized controlled studies on its effectiveness as a treatment for SARS-CoV-2 treatment.\(^{35,36}\) (ClinicalTrials.gov ID: NCT04292899, NCT04292730, NCT04257656, NCT04252664, and NCT04280705). (1) This drug has been demonstrated to inhibit SARS-CoV and MERS-CoV in vitro and in vivo experiments.\(^{37}\) (2) Data from compassionate use of remdesivir showed that 68\% of severe COVID-19 patients showed clinical improvement.\(^{38}\) (3) The first randomized controlled trial conducted at Hubei province of China showed that remdesivir was not associated with statistically significant clinical benefits including shortened time to clinical improvement.\(^{39}\) However, preliminary data from another randomized controlled trial in the USA, Korea, and other countries showed that remdesivir could shorten the time to recovery in adults hospitalized with COVID-19 and evidence of lower respiratory tract infection.\(^{40}\)
3) In randomized, open-label clinical trials comparing lopinavir/ritonavir with conservative treatment in patients with COVID-19, there was no clinical benefit of lopinavir/ritonavir and the trial was discontinued prematurely due to adverse drug reactions.\(^{40}\) In addition to negative results of the clinical trial, unfavorable pharmacodynamics of lopinavir/ritonavir and reported hepatotoxicity limits its use for treating COVID-19. (1) Lopinavir/ritonavir, a potent inhibitor of CYP3A4, can affect the metabolism of calcineurin inhibitors, sirolimus, and everolimus. (2) When using lopinavir/ritonavir, the dose of tacrolimus should be reduced by 1/20–1/50 folds.
4) Tocilizumab, a drug that targets the IL-6 receptor, is being tested only in hospitalized patients with moderate to severe COVID-19.\(^{41}\) Tocilizumab, a drug that targets the IL-6 receptor, is being tested only in hospitalized patients with moderate to severe COVID-19.\(^{41}\)
5) Hydroxychloroquine was confirmed to have an inhibitory effect on SARS-CoV-2 in vitro experiments.\(^{42}\) However, only non-randomized studies have been conducted in clinical trials with
Conflicting results. Further studies with well-designed clinical trials are needed to confirm the antiviral effect of hydroxychloroquine against COVID-19. (1) A combination therapy with hydroxychloroquine and azithromycin did not result in clinical improvement or viral clearance and several patients presented with prolonged QT intervals.

6) Several promising case reports of convalescent plasma transfusion have been reported in critically ill patients with COVID-19.

7) Niclosamide, an anti-helminthic drug, exhibited antiviral properties against SARS-CoV, MERS-CoV, and more recently SARS-CoV-2. Although niclosamide suffers a pharmacokinetic flaw of low adsorption, further development of its drug formulation could enable an effective delivery of this drug to the target tissue.

8) Clevudine, a nucleoside analogue developed in Korea that can inhibit the replication of hepatitis B virus, has recently demonstrated antiviral activity against SARS-CoV-2 and further clinical research is to be initiated. Attention to reversible myopathy, which has been reported in treated chronic hepatitis B patients due to the depletion of mitochondrial DNA leading to mitochondrial myopathy, is warranted.

9) ACE inhibitors and angiotensin receptor inhibitors (ARBs) can theoretically promote SARS-CoV-2 infection as they can increase the expression of ACE2, the target for the virus to enter cells. However, there has been insufficient evidence to limit ACEI/ARB treatment in COVID-19 patients because reports have shown that ACEI/ARB have cardio-pulmonary protective effects and increased expression of ACE2 can reduce acute lung injury.

[Considerations]

1. The use of lopinavir/ritonavir for the treatment of COVID-19 is not recommended.
2. Hydroxychloroquine with or without azithromycin is not generally recommended due to the possibility of serious side effects.
3. It is recommended that patients taking ACEI/ARB maintain the drug.

Healthcare workers’ protection and working environment

Healthcare workers and hospital staff are at risk of COVID-19 infection. Medical practitioners with confirmed infection of COVID-19 can spread the virus to patients.

Authors’ contribution

Manuscript preparation: Cho JY, Kim SS, Lee JH
Article reviews: Cho JY, Kim SS, Lee YS, Song DS, Lee JH, Kim JH
All authors revised and approved the final version of the manuscript.

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This document is approved by the Korean Association for the Study of the Liver (KASL). It is designed to provide information to clinicians on the treatment of patients with liver disease during the pandemic of COVID-19. The information provided in this document has not been subject to a heightened review to act as a standard of care or a practice guideline since new knowledge regarding the disease is continuously evolving. Management of liver diseases should be individualized according to each clinical situation and regional characteristics.

Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

Unmet needs of chronic hepatitis C in the era of direct-acting antiviral therapy

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The treatment of chronic hepatitis C (CHC) has been revolutionized in an era of all-oral direct-acting antivirals (DAAs) since 2014. Satisfactory treatment efficacy and tolerability can be provided by novel DAAs. Nevertheless, there are still some unmet needs and emerging issues in the treatment of CHC in the DAA era. Certain hard-to-cure populations are prone to have inferior treatment responses, including patients with severe liver decompensation, active hepatocellular carcinoma (HCC), and hepatitis C virus (HCV) genotype 3 (HCV-3) infection and those who experience multiple DAA treatment failures. Hepatitis B virus (HBV) reactivation during and after DAA treatment has raised concern regarding the use of prophylactic antivirals against HBV throughout DAA treatment. However, the standard strategy for the use of prophylactic antivirals is not uniform across regional guidelines. In the post-sustained virological response (SVR) period, HCC still occurs in a substantial proportion of patients. Due to the relatively short follow-up period, the net benefit of the achievement of an SVR by DAAs in the reduction of extrahepatic manifestations has not yet been determined. Attention must also be paid to HBV reinfection, particularly in high-risk populations. The most critical and unmet need for HBV elimination is the large gap in the HCV care cascade at the population level. To accomplish the World Health Organization’s (WHO) goal for HBV elimination by 2030, the expansion of access to HBV care requires a continuous effort to overcome practical and political challenges. (Clin Mol Hepatol 2020;26:251-260)

Keywords: Hepatitis C virus; Direct-acting antivirals; Treatment

INTRODUCTION

The treatment of chronic hepatitis C (CHC) has been revolutionized in an era of all-oral direct-acting antivirals (DAAs) since 2014. With the use of current novel DAAs, a sustained virological response (SVR) rate >95% can be attained in addition to satisfactory tolerability. Nevertheless, there are still some unmet needs and emerging issues in the treatment of CHC in the DAA era. Inferior treatment efficacies are observed in some hard-to-cure populations, including patients with severe liver decompensation, active hepatocellular carcinoma (HCC), and hepatitis C virus (HCV) genotype 3 (HCV-3) infection and those who experience multiple DAA treatment failures. Hepatitis B virus (HBV) reactivation during and after DAA treatment has raised concern regarding the use of prophylactic antivirals against HBV throughout DAA treatment.

Abbreviations:
APASL, Asian Pacific Association for the Study of the Liver; CHC, chronic hepatitis C; DAAs, direct-acting antivirals; EASL, European Association for the Study of the Liver; GLE, glecaprevir; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LDV, ledipasvir; MELD, Model for End-Stage Liver Disease; NAB, nonstructural protein 5A; NUC, nucleotide analog; NUCs, nucleotide analogs; PIB, pibrentasvir; RAS, resistance-associated substitutions; SOF, sofosbuvir; SVR, sustained virological response; VEL, velpatasvir; VOX, voxilaprevir; WHO, World Health Organization

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ures. Hepatitis B virus (HBV) reactivation during and after DAA treatment has raised concern for the use of prophylactic antivirals against HBV throughout the course of DAA treatment. However, there is no definite recommendation to guide which patients should be prescribed these prophylactic antivirals and for how long. In the post-SVR period, hepatocellular carcinoma (HCC) still occurs in a substantial proportion of patients, especially among those with advanced fibrosis and subjects who possess ongoing risk factors (e.g., diabetes, HBV dual infection, alcoholism). Due to the relatively short follow-up period, the net benefit of the achievement of an SVR by DAAs in the reduction of extrahepatic manifestations has not yet been settled. Attention must also be paid to HCV reinfection, particularly in high-risk populations. Last but not least, the most critical issue for HCV elimination is the large gap in the HCV care cascade at the population level. The abovementioned issues will be highlighted and discussed in the current review (Table 1).

**DIFFICULT-TO-CURE POPULATIONS**

**HCV-3**

HCV-1 is considered a difficult-to-cure genotype in the interferon era. In the landscape of the DAA era, the treatment response of HCV-1 infection is no longer suboptimal. Rather, a relatively lower SVR rate could be observed in the treatment of HCV-3 infection, particularly in patients with liver cirrhosis who failed prior antivirals. Ironically, patients with HCV-3 infection are prone to have advanced liver disease at the time of presentation. In the ASTRAL-3 study, a high SVR12, defined as undetectable HCV RNA throughout 12 weeks of the posttreatment follow-up period, of 95% could be attained in HCV-3 infected patients treated with sofosbuvir (SOF) plus velpatasvir (VEL). However, the SVR rate was only 89% in the subgroup of treatment-experienced patients with cirrhosis. While the pooling analysis of phase III studies of SOF/VEL revealed an SVR rate of 93% in patients with nonstructural protein 5A (NS5A) resistance-associated substitutions (RASs), a lower SVR rate of 84% was denoted in patients with the Y93H mutation in the ASTRAL-3 study. This finding may prompt RAS testing or the addition of ribavirin to the regional guidelines for the treatment of patients with cirrhosis with HCV-3 infection receiving SOF/VEL. Beyond the impact of RASs, HCV-3 subtyping may also account for treatment inferiority. In a phase III study using SOF/VEL for 12 weeks in Asia, an SVR rate of only 76% was noted in HCV-3b-infected patients. Among them, an SVR rate of 89% in patients without cirrhosis but only 50% in patients with cirrhosis patients was depicted. For the other pangenotypic DAA regimen, glecaprevir (GLE)/pibrentasvir (PIB), an SVR rate of 94.9% could be attained in HCV-3 treatment-naïve patients without cirrhosis who received 8 weeks of treatment in the ENDURANCE-3 study. Among them, the SVR rate of patients with the A30K mutation in NS5A was 75% (12/16) compared to 99% (135/137) in those without the mutation. As HCV-3 treatment-naïve compensated patients with cirrhosis could be allocated to abbreviated 8-week GLE/PIB regimen with an SVR rate of 98.4% (60/61) in the per-protocol analysis of the EXPEDITION-8 study, interferon/ribavirin- or SOF (PRS)-experienced patients should remain on a 12- to 16-week regimen of GLE/PIB to ensure treatment efficacy.

**Liver decompensation**

The portal-systemic-shunting-related poor first-pass effect and bioavailability of DAAs may result in the suboptimal antiviral response in patients with liver decompensation. SOF plus NS5A inhibitor-based therapy is recommended since protease inhibitors are contraindicated. In the SOLAR-1 and SOLAR-2 studies, the SVR12 rate was 87% and 85–86% in Child-Pugh class B and
Child-Pugh class C patients after 12 weeks of SOF/ledipasvir (LDV) treatment, respectively.\textsuperscript{11,12} In the ASTRAL-4 study, although the treatment efficacy could be improved up to 94\% in Child-Pugh class B patients who were allocated to 12 weeks of SOF/VEL plus ribavirin treatment, the SVR12 rate was as low as 85\% in patients with HCV-3-infection.\textsuperscript{13} Adding ribavirin to DAAs in the population is warranted to ascertain treatment efficacy. The role of ribavirin in the improvement in DAA efficacy is not fully understood,\textsuperscript{14} and whether it improves early kinetics and promotes immune modulations to diminish the chance of relapse following viral mutation awaits further identification.\textsuperscript{15} It should be noted that the failure to attain an SVR in a significant proportion of decompensated patients was due to adverse events or mortality-related treatment discontinuation rather than to virological failure in the trials,\textsuperscript{11-13} which reflected the difficulty in the management of the fragile subjects in the clinical setting. For example, a recent study using SOF/VEL plus ribavirin in the treatment of 23 patients with Child-Pugh class C resulted in an SVR12 of only 70\% (16/23) in an intention-to-treat analysis. The lack of assessment in six of the seven patients during the study period was due to mortality not related to the study drugs.\textsuperscript{16}

Apart from the issue of treatment efficacy, ongoing deterioration of the Model for End-Stage Liver Disease (MELD) score was noted in 17–43\% of Child-Pugh class B and 11–18\% of Child-Pugh class C patients, even after the eradication of HCV.\textsuperscript{17} The timing of DAA initiation before or after liver transplantation in decompensated patients on the waiting list increases the complexity when donor feasibility is taken into account. It is recommended that patients whose MELD score is >18–20 should be considered for liver transplantation first if the local situation allows and if the donor is available within 6 months.\textsuperscript{4,18} On the other hand, although patients could be delisted due to an improvement in liver function reserve by DAAs before liver transplantation, this may occur in only a limited subset of patients.\textsuperscript{19} Furthermore, re-decompensation,\textsuperscript{20} a loss of transplantation priority with the occurrence of HCC, and a poor quality of life (so-called MELD purgatory)\textsuperscript{21} become challenging tasks clinicians must face in the post-SVR period.

**Multiple DAA treatment failures**

For patients with previous DAA treatment failures, 12-week SOF/VEL/voxilaprevir (VOX) and 24-week SOF/VEL plus ribavirin treatment for patients with HCV-1-6 infection have been approved.\textsuperscript{12,24} For HCV-1 infected patients, 12-week GLE/PIB treatment for prior NS3/4A protease inhibitor-experienced patients and 16-week GLE/PIB treatment for prior NS5A inhibitor-experienced patients have also been approved.\textsuperscript{25} Recently, 179 DAA-experienced patients were treated with SOF/VEL/VOX with or without RBV in the real-world setting. Of them, 82\% of patients carried a RAS in the NS3, NS5A or NS5B regions before retreatment. The overall SVR12 rate was 96\% in a per-protocol analysis. Nevertheless, patients with liver cirrhosis (91\%, 71/78) and HCC (71\%, 5/7) had a significantly lower SVR rate. Due to overlapping antiviral classes, a relatively low SVR rate of 88.4\% (23/26) was noted in SOF/VEL-experienced patients who received SOF/VEL/VOX,\textsuperscript{26} indicating that the retreatment of prior-DAA-failed patients should be managed sophisticatedly and on an individual basis.

Certain unapproved strategies with multitarget regions have also been adopted with satisfactory efficacy.\textsuperscript{27,28} The timing and necessity of RAS testing remain elusive.\textsuperscript{4,5} Currently, the treatment strategy for “very difficult-to-cure” patients, who have been defined as patients with NS5A RASs who failed twice to achieve an SVR after a combination regimen including a protease inhibitor and/or an NS5A inhibitor, remains unclear. The European Association for the Study of the Liver (EASL) guideline has advocated the use of SOF/VEL/VOX or SOF/GLE/PIB in addition to ribavirin and has extended the treatment duration to 16–24 weeks. Nevertheless, the recommendation awaits validation by prospective studies (Table 2).

Finally, since protease inhibitors are contraindicated for decompensated patients, the only recommended regimen is the combination of SOF/VEL+RBV for 24 weeks. However, the SVR rate might be suboptimal in patients with baseline NS5A RASs and those who could not tolerate full course ribavirin. This highlights the urgent need for early identification and treatment for CHC patients to avoid progression of liver disease to decompensated cirrhosis.\textsuperscript{29}

**Patients with active HCC**

Treatment efficacy in patients with HCC has been widely discussed (Fig. 1). Compared to patients without HCC or with inactive HCC, whether patients with active HCC have an inferior treatment response remains controversial.\textsuperscript{20,30} A recent meta-analysis of 49 studies showed that the SVR rate was significantly lower in patients with active HCC (73.1\%) than in those with inactive HCC (92.6\%) or without HCC (93.3\%).\textsuperscript{31} The argument exists that in these studies, there were unequal patient and viral characteristics as well as suboptimal regimens in early studies, which may end in
Table 2. Clinical trials of DAA rescue therapy in patients with prior DAA treatment failure*

<table>
<thead>
<tr>
<th>Trial</th>
<th>Study design</th>
<th>Prior DAAs</th>
<th>GT</th>
<th>Salvage DAA</th>
<th>Duration (weeks)</th>
<th>SVR12 (%)</th>
<th>n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>POLARIS-1 (2017)</td>
<td>Phase III, double-blind, multicenter, randomized controlled trial</td>
<td>NS5A+NS3±NS5B1 (32%); NS5A+NS5B1 (61%); NS5A (7%)</td>
<td>1–6</td>
<td>SOF/VEL/VOX</td>
<td>12</td>
<td>96 (253/263)</td>
<td></td>
</tr>
<tr>
<td>POLARIS-4 (2017)</td>
<td>Phase III, multicenter, randomized, open-label study</td>
<td>NS5B1+NS3I (25%); NS5B1 (72%)</td>
<td>1–4</td>
<td>SOF/VEL/VOX</td>
<td>12</td>
<td>98 (178/182)</td>
<td></td>
</tr>
<tr>
<td>POLARIS-4 (2017)</td>
<td>Phase III, multicenter, randomized, open-label study</td>
<td>NS5B1+NS3I (25%); NS5B1 (72%)</td>
<td>1–3</td>
<td>SOF/VEL</td>
<td>12</td>
<td>90 (136/151)</td>
<td></td>
</tr>
<tr>
<td>GS-US-342-1553 (2017)</td>
<td>Phase II, open-label study</td>
<td>SOF/VEL (39%); SOF/VEL+RBV (20%); SOF/VEL/VOX (41%)</td>
<td>1–3</td>
<td>SOF/VEL+RBV</td>
<td>24</td>
<td>91 (63/69)</td>
<td></td>
</tr>
<tr>
<td>C-SWIFT (2017)</td>
<td>Phase II, open-label, single-center, multiple-arm study</td>
<td>EBR/GZR+SOF (100%)</td>
<td>1</td>
<td>EBR/GZR+SOF+RBV</td>
<td>12</td>
<td>100 (23/23)</td>
<td></td>
</tr>
<tr>
<td>CERTAIN-1 (2018)</td>
<td>Phase III, open-label, multicenter study</td>
<td>DCV+ASV (91%); PegIFN/RBV+SMV (6%); SOF+RBV (3%)</td>
<td>1, 2</td>
<td>GLE/PIB</td>
<td>12</td>
<td>94 (31/33)</td>
<td></td>
</tr>
<tr>
<td>ANRS HC34 REVENGE (2018)</td>
<td>Phase II, open-label study</td>
<td>SOF+DCV (29%); SOF/LDV (64%); SOF+SMV (7%)</td>
<td>1, 4</td>
<td>EBR/GZR+SOF+RBV</td>
<td>16/24</td>
<td>96 (25/26)</td>
<td></td>
</tr>
<tr>
<td>Izumi et al. (2018)</td>
<td>Phase III, multicenter, open-label study</td>
<td>NS5A+NS3±NS5B1 (79%); NS5B1±NS3I (16%); NS5A±NS5B1 (5%)</td>
<td>1, 2</td>
<td>SOF/VEL+RBV</td>
<td>12</td>
<td>82 (47/57)</td>
<td></td>
</tr>
<tr>
<td>Izumi et al. (2018)</td>
<td>Phase III, multicenter, open-label study</td>
<td>NS5A+NS3±NS5B1 (72%); NS5B1±NS3I (15%); NS5A±NS5B1 (13%)</td>
<td>1, 2</td>
<td>SOF/VEL+RBV</td>
<td>24</td>
<td>97 (58/60)</td>
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<tr>
<td>MAGELLAN-1 (part 2) (2018)</td>
<td>Phase III, multicenter, randomized, open-label study</td>
<td>NS3/4A PI (32%); NS5A (36%); N3/4A PI+NS5A (32%)</td>
<td>1, 4</td>
<td>GLE/PIB</td>
<td>12</td>
<td>89 (39/44)</td>
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<tr>
<td>MAGELLAN-1 (part 2) (2018)</td>
<td>Phase III, multicenter, randomized, open-label study</td>
<td>NS3/4A PI (28%); NS5A (38%); N3/4A PI+NS5A (34%)</td>
<td>1, 4</td>
<td>GLE/PIB</td>
<td>16</td>
<td>91 (43/47)</td>
<td></td>
</tr>
<tr>
<td>RESOLVE (2019)</td>
<td>Phase IIb, multicenter, open-label study</td>
<td>LDV/SOF (89%); PrOD (4%); DCV/ASV (4%); EBR/GZR (3%); SMV+SOF (3%); DCV+SOF (1%); SOF/VEL (1%)</td>
<td>1</td>
<td>SOF/VEL/VOX</td>
<td>12</td>
<td>91 (70/77)</td>
<td></td>
</tr>
<tr>
<td>MAGELLAN-3 (2019)</td>
<td>Phase IIb, open-label, nonrandomized, multicenter study</td>
<td>GLE/PIB (100%)</td>
<td>1–3</td>
<td>GLE/PIB+SOF+RBV</td>
<td>12/16</td>
<td>96 (22/23)</td>
<td></td>
</tr>
<tr>
<td>Lok et al. (2019)</td>
<td>Phase IIb, open-label, randomized, study</td>
<td>SOF/LDV (95%); SOF/VEL (5%)</td>
<td>1, non-LC</td>
<td>GLE/PIB</td>
<td>12</td>
<td>90 (70/78)</td>
<td></td>
</tr>
<tr>
<td>Lok et al. (2019)</td>
<td>Phase IIb, open-label, randomized, study</td>
<td>SOF/LDV (92%); SOF/VEL (6%); SOF+DCV (2%)</td>
<td>1, non-LC</td>
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<tr>
<td>Lok et al. (2019)</td>
<td>Phase IIb, open-label, randomized, study</td>
<td>SOF/LDV (100%)</td>
<td>1, LC</td>
<td>GLE/PIB+RBV</td>
<td>12</td>
<td>86 (18/21)</td>
<td></td>
</tr>
<tr>
<td>Lok et al. (2019)</td>
<td>Phase IIb, open-label, randomized, study</td>
<td>SOF/LDV (90%); SOF/VEL (10%)</td>
<td>1, LC</td>
<td>GLE/PIB</td>
<td>16</td>
<td>97 (28/29)</td>
<td></td>
</tr>
</tbody>
</table>

DAA, direct-acting antiviral; GT, genotype; SVR, sustained virological response; NS, nonstructural; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir; RBV, ribavirin; EBR, elbasvir; GZR, grazoprevir; DCV, daclatasvir; ASV, asunaprevir; PegIFN, pegylated interferon; SMV, simeprevir; GLE, glecaprevir; PIB, pibrentasvir; LDV, ledipasvir; PI, protease inhibitor; PrOD, paritaprevir/ritonavir/ombitasvir+dasabuvir; LC, liver cirrhosis.

*Adapted from 2020 Taiwan Consensus Statement on the Management of Hepatitis C.
different treatment responses between groups. To overcome this pitfall, Ogawa et al. conducted a propensity-score-matched study for age, sex, cirrhosis, prior treatment, HCV genotype, treatment regimen, baseline platelet count, HCV RNA, total bilirubin, alanine aminotransferase, and albumin level to evaluate the treatment outcome in a large Asian cohort with or without HCC. Patients with active HCC (85.5%) but not those with inactive HCC (93.7%) had a lower SVR rate than those without HCC (95.0%; adjusted odds ratio, 0.28; \( P = 0.01 \)).

A significantly improved survival benefit has been observed in early-stage HCC patients who received curative cancer treatment followed by DAA therapy. If liver function does not compromise cancer treatment (e.g., as in the case of the ability to undergo anesthesia for surgical resection), HCC with curative potential should be treated first before antiviral therapy to ensure a higher chance of treatment success. On the other hand, the timing of the initiation of DAAs in patients with incurable HCC becomes a challenging issue since residual HCC may compromise DAA treatment efficacy. Patients with active HCC may have more safety concerns during DAA treatment. In addition, with the breakthrough innovations of systemic therapy in advanced HCC, whether the treatment with DAAs and immunotherapy/target therapy alter the clinical outcome remains unknown. Furthermore, the most critical point is whether HCV eradication will prolong long-term survival in patients with incurable HCC. Dang et al. conducted a propensity-matched study enrolling 1,239 untreated patients and 437 patients with an SVR. Among them, 70.6% of patients received curative HCC treatment, whereas the remaining 29.4% received palliative treatment. The results demonstrated that the attainment of an SVR significantly reduced 5-year all-cause mortality and liver relative mortality in both groups, indicating that treatment should not be withheld from those who are not eligible for curative HCC therapy. One of the postulated reasons is that the recovery or preservation of liver function after HCV eradication may offer chances for repeated cancer therapy. Nevertheless, patient numbers in certain subpopulations were too limited for the conclusions to be generalized. Upon closer look, patients with Child-Pugh class B did not benefit much from HCV eradication in terms of the long-term outcome, whereas the achievement of an SVR did not provide survival benefits in patients with Child-Pugh class C. Unlike decompensated patients without HCC, the deteriorated liver function in HCC patients may be due to cancer burden, which also precludes them from liver transplantation. The role of HCV eradication in the terminally ill population remains an unknown domain for exploration.

HBV REACTIVATION IN PATIENTS WITH DUAL HCV/HBV INFECTION

The issue of HBV reactivation after HCV viral suppression deserves more attention, particularly in HBV hyperendemic areas. A meta-analysis of 17 studies observed that the rate of HBV activation was 24% in chronic hepatitis B patients, which in turn led to 9% clinical hepatitis. A total of 1.4% of patients with resolved HBV experienced HBV reactivation, but none had clinical relapse. Until now, no prospective controlled study has denoted which, when, or for how long HBV patients should receive prophylactic nucleoside/nucleotide analogs (NUCs) or monitoring during/post-DAA therapy. The recommendations of regional guidelines are largely based on clinical rationality, and the level of evidence is not strong enough to draw conclusions. In addition, there are somewhat different viewpoints among regional guidelines. For example, the EASL suggests that patients who are hepatitis B virus surface antigen (HBsAg)-positive should receive prophylactic nucleoside/nucleotide analogs (NUCs) or monitoring during/post-DAA therapy. The Asian Pacific Association for the Study of the Liver (APASL) suggests pre-emptive NUC prophylaxis in HBV patients with advanced liver disease or pre-existing HCC. For patients with mild liver disease, close monitoring without anti-HBV therapy is another option. When to stop NUCs should follow APASL HBV guidelines. One of the best ways to monitor these patients is to identify a surrogate marker that can positively and negatively predict HBV reactivation. Factors predictive of HBV flares have been detectable HBV DNA before DAA therapy, alanine aminotransferase >2 times the upper limit of normal at baseline, and HBsAg >10 IU/mL at baseline.
when using DAAs or baseline HBV DNA >300 IU/mL when using interferon-based therapy. However, the surrogates were just identified as statistically significant in distinguishing events between groups, and their accuracies in guiding clinical decisions need to be validated by prospective studies. HBV-related clinical hepatitis may lead to severe decompensation and even mortality. From this viewpoint, patients with advanced liver fibrosis should at least receive HBV prophylaxis before and during DAA therapy. The treatment duration and follow-up strategy after the discontinuation of NUCs awaits further clarification.

**POST-SVR ERA**

**HCC**

Similar to interferon-based therapy, the achievement of an SVR by DAAs decreases the risk of HCC occurrence and does not increase the risk of HCC recurrence. Notably, the benefits of the achievement of an SVR in the reduction of HCC risk in decompensated patients are controversial. Again, this controversy raises the importance of the discussion of the ideal timing for liver transplantation in the treatment of decompensated patients mentioned earlier. Several factors have been predictive of HCC in the post-SVR period. The developed prediction model for HCC has also been created to guide follow-up strategies. It is noteworthy that the risk of HCC persists even after 10 years of viral eradication. It has been suggested that HCV-induced oncogenic effects are elicited before treatment and that the “epigenetic scar” may leave and persist long after viral eradication, leading to a lifelong risk of HCC. The EASL advocates that patients with an SVR could be discharged if they do not possess advanced fibrosis or other comorbidities. The recommendation may be based on the cost-effectiveness of surveillance. Nevertheless, HCC still occurs in so-called low-risk patients after viral eradication. As the updated APASL guidelines recommended the follow-up of patients with an SVR at different intervals based on their underlying risks, the surveillance of HCC, particularly low-risk patients, in the post-SVR period should be judged on a case-by-case basis including local medical accessibility and feasibility.

**Extrahepatic manifestations**

HCV eradication may also improve long-term extrahepatic manifestations. Due to the short follow-up period of the post-DAA period and low incidences of index outcomes, the benefits of DAAs in the reduction in extrahepatic complications are not universally granted. A retrospective cohort study has shown that the risk of non-Hodgkin lymphoma was not significantly reduced after DAA-induced SVR (hazard ratio, 0.86; 95% confidence interval, 0.52–1.43) after only 2.01 years of follow-up. HCV eradication may reduce the risk of vascular events. However, this was not always the case. A study comprising 160,875 subjects showed that the risk of coronary heart disease and stroke did not differ between patients with and without an SVR. Hypolipidemia during viremic status can be reversed to deteriorate lipid profiles after DAA therapy. An increase in the small dense low-density lipoprotein cholesterol level corresponding to increased carotid intimamedia thickness 1 year after DAA therapy has been reported. Attention should be paid not only to liver-related complications but also to the extrahepatic consequence of cardio-cerebrovascular disease in the post-SVR period.

**Reinfection**

The reinfection rate after HCV eradication should be rare in the general population. Nevertheless, reinfection has been frequently encountered in patients with high-risk behaviors. A systemic review showed that the HCV reinfection rate 5 years after HCV eradication was 0.95% in a low-risk population, 10.7% in a high-risk population (prisoners and patients who inject drugs), and up to 15.0% in subjects with HCV and human immunodeficiency virus (HIV) coinfection. Due to the ease of access and the fact that more treatment candidates are allowed in the DAA era, it is postulated that more HCV reinfection may occur. An annual incidence of up to 5.9% per person-year has been reported in HIV/HCV coinfected men who have sex with men after DAA therapy. Multidisciplinary approaches to high-risk populations should be adopted, which include health counseling for safe sex, harm reduction services, opioid substitution therapy and so forth. Treatment as prevention and an increase in the treatment uptake rate followed by a decrease in viral reservoirs at the population level are the most critical policies to be applied.

**GAP TO ACHIEVE HCV ELIMINATION**

The WHO has set ambitious goals for the control of viral hepatitis by 2030. However, only a few countries are on track for HCV elimination. In the DAA era, where treatment efficacy and tolera-
bility are no longer the major concern, the identification of multiple barriers that exist in patients, providers and institutions that prevent the delivery of HCV care is the most difficult task. A comprehensive HCV care cascade involves blood safety and infection control, harm reduction, proper screening for unawareness, accurate and efficient diagnosis, and linking to medical care. To scale up prevention and treatment, outreach screening programs would be the key determinant for HCV elimination. Several outreach screening programs and treatment strategies with the concept of microelimination in hyperendemic areas and high-risk populations have been adopted in Taiwan.

CONCLUSIONS

By using multtarget DAA therapy with high genetic barriers, adding ribavirin and extending treatment duration, difficult-to-cure patients may no longer be difficult to cure. The long-term benefit of HCV eradication in decompensated and/or active HCC patients remains elusive. Since it is impractical to conduct a prospective untreated-comparator study, larger observational studies comprising diverse patient characteristics with a longer follow-up period are warranted to judge the impact of SVR. For subjects with HBV/HCV dual infection who do not fulfill the indications for anti-HBV therapy before DAA treatment, the addition of prophylactic NUCs is practical and essential. Further studies that explore the pathophysiological interaction of the two viruses may help to identify the candidates for and strategy of NUC prophylaxis. Much work remains to be done with many barriers standing in the way of achieving the WHO goal of HCV elimination. Each step may require the support and engagement of the local healthcare system, infrastructural commitment and nongovernment organizations. The expansion of access to HCV care requires a continuous effort to overcome the practical and political challenges.

Authors’ contribution

CFH and MLY contributed to the design and writing of the manuscript.

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CONFLICTS OF INTEREST

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Chung-Feng Huang: Speaker for AbbVie, Abbott, BMS, Gilead, and Merck.

REFERENCES


43. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. J Hepatol 2017 Sep 5. [Epub ahead of print]


64. Razavi H. Mathematical modelling: treatment-as-prevention. The 4th International Symposium on Hepatitis in Substance Users (INHSU 2015); 2015 Oct 7-9; Sydney, Australia.


Hepatitis B virus (HBV) cannot be eliminated completely from infected hepatocytes because of the presence of intrahepatic covalently closed circular DNA (cccDNA). As chronic hepatitis B (CHB) can progress to cirrhosis and hepatocellular carcinoma (HCC), it is important to manage CHB to prevent HCC development in high-risk patients with high viral replicative activity or advanced fibrosis. Serum biomarkers are noninvasive and valuable for the management of CHB. Hepatitis B core-related antigen (HBcrAg) correlates with serum HBV DNA and intrahepatic cccDNA. In CHB patients with undetectable serum HBV DNA or loss of HBsAg, HBcrAg still can be detected and the decrease in HBcrAg levels is significantly associated with hopeful outcomes. Therefore, HBcrAg can predict HCC occurrence or recurrence. Measurement of the Mac-2 binding protein glycosylation isomer (M2BPGi) has been introduced for the evaluation of liver fibrosis. Because elevated M2BPGi in CHB is related to liver fibrosis and the prediction of HCC development, monitoring its progression is essential. Because alpha fetoprotein (AFP) has insufficient sensitivity and specificity for early-stage HCC, a combination of AFP plus protein induced by vitamin K absence factor II, or AFP plus \textit{Lens culinaris} agglutinin-reactive fraction of alpha-fetoprotein might improve the diagnosis of HCC development. Additionally, Dickkopf-1 and circulating immunoglobulin G antibodies are the novel markers to diagnose HCC or assess HCC prognosis. This review provides an overview of novel HBV biomarkers used for the management of intrahepatic viral replicative activity, liver fibrosis, and HCC development. (\textit{Clin Mol Hepatol} 2020;26:261-279)

\textbf{Keywords:} Hepatitis B core-related antigen; Mac 2-binding protein glycan isomer; Alpha-fetoprotein; \textit{Lens culinaris} agglutinin-reactive fraction of alpha-fetoprotein; Protein induced by vitamin K absence or antagonist-II

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  \item Hepatitis B core-related antigen (HBcrAg) correlates with serum HBV DNA and intrahepatic cccDNA.
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INTRODUCTION

Hepatitis B virus (HBV) is a common cause of acute and chronic liver disease. The World Health Organization estimates that in 2015, 257 million people suffered from chronic hepatitis B (CHB), defined as hepatitis B surface antigen (HBsAg)-positive. The majority of new HBV infections occur in highly endemic areas such as China, Southeast Asia, and sub-Saharan Africa. HBV has been the most common cause of hepatocellular carcinoma (HCC), being responsible for >50% of cases worldwide. Compared with uninfected patients, the relative risk of HCC development increases 15–20-fold in patients with HBV infection. Meanwhile, Ho et al. reported the clinical presentations of HCC have significantly changed over the past 12 years. In their report, hepatitis B and C virus-associated HCC became less common, and more patients were diagnosed at early cancer stage.

Unfortunately, although nucleos(t)ide analogues (NAs) or interferon (IFN) can efficiently suppress HBV replication, these are not curative treatments. These drugs do not directly target covalently closed circular DNA (cccDNA), the key molecule responsible for intrahepatic viral persistence. cccDNA is a stable, extra-chromosomal transcriptional template for all HBV mRNAs such as pregenomic RNA. The amount and transcriptional activity of cccDNA in hepatocytes is important for CHB progression and clinical outcomes. The current aim of anti-HBV treatment is primarily to suppress complications associated with progressive inflammation and fibrosis, i.e., liver failure and decompensated cirrhosis.

HCC diagnosis and surveillance are mostly based on the detection of tumor markers and imaging techniques. There is still a requirement for more reliable, non-invasive, and cost-effective biomarkers for CHB management. Especially, a significant number of CHB patients with non-cirrhotic liver develop HCC. Current guidelines advise 6-monthly abdominal ultrasound surveillance for HCC in advanced fibrosis or cirrhotic CHB patients and in non-cirrhotic patients depending on ethnic background and age. Therefore, identifying biomarkers to better predict or diagnose HCC is critical.

Figure 1. The relationship between chronic HBV infection and liver disease progression. The figure shows the clinical stages involved in the natural history of CHB. The serum biomarkers HBcrAg and M2BPGi provide valuable predictive data for the effective management of CHB. It is important to monitor patients at high risk and to treat them early to prevent liver complications, cirrhosis, and HCC development. AFP, PIVKA-II, and AFP-L3 are HCC-specific tumor markers summarized in this review. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization; TKIs, tyrosine kinase inhibitors; AFP, alpha fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; DKK-1, Dickkopf-1; HBcrAg, hepatitis B core-related antigen; M2BPGi, Mac-2 binding protein glycan isomer; HBsAg, hepatitis B surface antigen.
HCC remains an important clinical and research priority. In this review, we introduce novel biomarkers with great potential for CHB management and prognostic evaluation. The first is a surrogate marker of intrahepatic HBV replication, hepatitis B core-related antigen (HBcrAg), which has revealed a good correlation with cccDNA.\(^{17}\) The second, Mac-2-binding protein glycosylation isomer (M2BPGi), is a liver fibrosis marker that might predict HCC development.\(^{18}\) Finally, the third is a tumor marker, alpha-fetoprotein (AFP), a protein induced by vitamin K absence or antagonist-II (PIVKA-II), Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), Dickkopf-1 (DKK-1) and circulating immunoglobulin G (IgG) antibodies. We will focus on the clinical utility of these markers as predictors of HBV-related HCC development (Fig. 1).

**MONITORING OF BIOMARKERS RELATED TO HBV INFECTION**

With recent developments in molecular research methods, several biomarkers associated with the natural history of CHB and effectiveness of antiviral therapy have been identified.\(^{17,19}\) Conventional serological biomarkers include serum HBV DNA levels and HBsAg titers, both of which predict the risk of cirrhosis and HCC.\(^{20,21}\) Quantitation of HBsAg has been used as a predictive marker of liver disease, spontaneous HBsAg seroclearance, cirrhosis, and HCC development, when complementary to the measurement of HBV DNA.\(^{22-25}\) However, cirrhosis and HCC can still occur in patients with undetectable HBV DNA\(^{26}\) and HBsAg seroclearance.\(^{27,28}\) Additionally, essential information of innovative and effective biomarkers including prediction of spontaneous or treat-

![Figure 2](image-url). The lifecycle of HBV and HBcrAg. The schematic shows the steps involved in the lifecycle of HBV. Importantly, it shows the sources of various HBV-related molecules used routinely for diagnosis, clinical monitoring, and prognosis of HBV infection. These include the core-related antigens (p22cr, HBe, and core antigens), HBsAg, and HBV DNA measured in serum. cccDNA, although measurable in serum in association with liver injury, is mainly measured from liver biopsy samples. HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B envelope antigen; HBV, hepatitis B virus.
ment-induced hepatitis B envelope antigen (HBeAg) seroconversion, and responses before/after cessation of NA treatment are still required.

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

We recently described the clinical application of a new effective biomarker, HBcrAg, including the association of HBcrAg with other biomarkers, its competency for the prediction of clinical outcomes, and its role as a predictor of treatment and anti-HBe in the test samples.¹⁰ Japan was first to recommend HBcrAg in clinical guidelines for CHB management followed by the greater Asian region and then Europe.¹¹,³⁸,³⁹

**Recent clinical assessments of HBcrAg**

The novel biomarker, HBcrAg, has been used to support monitoring of CHB and the prediction of clinical outcomes. In this section, we describe briefly the recently reported clinical applications of HBcrAg (Table 1).

Serum HBcrAg levels are closely associated with intrahepatic cccDNA levels, as well as serum HBV DNA.³⁸,⁴⁰ Riveiro-Barciela et al.³⁹ reported that HBsAg levels <3 log IU/mL were only valuable for detecting genotype D HBV inactive carriers. A single HBcrAg measurement ≤3 log U/mL plus HBV DNA ≤2,000 IU/mL was highly accurate in detecting HBV inactive carriers, regardless of their HBV genotype.³⁹ Testoni et al.²⁸ recently confirmed serum HBcrAg levels were significantly higher in HBeAg-positive patients than in HBeAg-negative patients without antiviral treatment and that they correlated with serum HBV DNA, intrahepatic HBV DNA, pgRNA and cccDNA levels, in addition to transcriptional activity. Patients negative for HBcrAg (<3 log U/mL) had lower amounts of intrahepatic cccDNA and lower cccDNA activity than HBcrAg-positive patients.³⁸ Hasegawa et al.⁴¹ created a helpful prediction model for intrahepatic cccDNA levels in CHB patients. The method was named the fasting blood sugar (FBS)–cres score based on the variables used (FBS, HBcrAg, HBeAg, and HBsAg). The FBS-cres score is calculated by the following equation: 3.1686 – (0.0148 × FBS) + (0.1982 × HBcrAg) + (0.0008168 × HBeAg) + (0.1761 × HBsAg)). For example, in the training cohort, a significant correlation was shown between HBcrAg and cccDNA levels (P<0.0001, r=0.67), whereas the FBS-cres score was more closely correlated to cccDNA level (P<0.0001, r=0.81).⁴¹

In resource-limited areas such as Africa, HBV DNA quantification assays have limited availability and are expensive. Shimakawa et al.⁴² evaluated the prospect of HBcrAg to identify Gambian patients qualified for treatment, using a new experimental algorithm that does not include HBV DNA. An easy treatment algorithm us-
ing HBcrAg without HBV DNA showed high area under the receiver operating characteristics (AUROC) (0.91; 95% confidence interval [CI], 0.88–0.95), with a sensitivity of 96.6% and specificity of 85.8%. The measurement of HBcrAg, which is 5–10 times less expensive per test than the quantification of HBV DNA, might replace HBV DNA testing.\(^4\)

**Change in HBcrAg and other HBV markers under NA therapy**

A reduction in serum HBV DNA does not correlate with a decrease of intrahepatic cccDNA in patients receiving anti-HBV therapy. In a study of 43 patients treated with NA (median, 126 months) who are positive or negative for HBeAg, although 51% still had detectable intrahepatic cccDNA.\(^5\) Similar findings were

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<th>Table 1. Clinical applications of HBcrAg in CHB patients</th>
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HBcrAg, hepatitis B core-related antigen; CHB, chronic hepatitis B; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; PEG-IFN, pegylated interferon; NA, nucleos(t)ide analogue; LAM, lamivudine; ETV, entecavir; HCC, hepatocellular carcinoma; BCP, basal core promoter.
reported in 24 patients positive for HBeAg treated with sequential therapy of pegylated interferon (PEG-IFN) and adenovir (ADV) followed by ADV monotherapy—46% and 66% patients had undetectable serum HBV DNA and detectable intrahepatic cccDNA, respectively.44

The difference between serum HBcrAg and HBV DNA can be explained by the action of NA on reverse transcription and the subsequent prevention of HBV DNA replication, while HBcrAg production remains persistently. The reduction of HBcrAg demonstrated a good correlation with the magnitude of change in intrahepatic cccDNA levels.45,46 In contrast to serum HBV DNA, the reduction of HBcrAg was slower during NA treatment, with an increase in the ratio of serum HBcrAg:HBV DNA after 3 months of lamivudine.33 Furthermore, in patients receiving NA treatment with undetectable serum HB DNA, 78% had persistent HBcrAg.46 Even in patients with documented HBsAg seroclearance, 21% had detectable serum HBcrAg, in contrast to detectable serum HBV DNA in only 2.1%.47,48

Serum HBcrAg levels at baseline and changes while on anti-HBV therapy may also predict suitable indicators in CHB patients. Because HBV DNA is not detectable in most patients receiving NAs, HBcrAg will be used as a surrogate marker. For patients positive for HBeAg treated with PEG-IFN, a high baseline HBcrAg level >8 log U/mL conferred a >94.4% negative predictive value (NPV) for achieving HBeAg seroconversion and suppression of HBV DNA at 12 weeks (n=46).49 Furthermore, HBcrAg levels changed by treatment might predict clinical outcomes. In 58 patients positive for HBeAg treated with PEG-IFN, HBcrAg levels at week 12 predicted HBeAg seroconversion at 24 weeks after finishing treatment with an AUROC of 0.896.50 For patients treated with NA therapy (n=39), HBcrAg levels were lower in patients with NA-induced HBeAg seroconversion compared with patients who remained HBeAg-positive.45

As an advanced technique, a combination of HBcrAg and HBsAg assays may be used to identify patients who are unlikely to achieve treatment end-points, i.e., HBeAg seroconversion.11 Wang et al.51 identified predictors of seroconversion using serum quantitative HBsAg and HBcrAg, in HBeAg-positive patients. Data and samples were obtained from 118 HBeAg-positive adults with HBV genotypes A–G treated with NA. Approximately 36.4% of patients achieved HBeAg seroconversion after NA treatment (median, 39 months). Regarding the treatment kinetics of HBV DNA, HBsAg and HBcrAg differed between patients with and without HBeAg seroconversion.51

Additionally, baseline HBcrAg level is an independent predictor of long-term HBcrAg below the limit of detection.52 Wang et al.52 investigated the long-term kinetics of serum HBcrAg and its correlation with serum HBsAg in CHB patients positive or negative for HBeAg with NA therapy over 8 years. At 8 years from the start of NA treatment, 21.3% of patients achieved a serum HBcrAg level of <3 log10 U/mL, and only baseline HBcrAg was an independent predictor.52

**HBcrAg in the prediction of NA cessation point**

Once seroconversion to anti-HBe occurs, the reduction of HBcrAg and HBsAg followed by HBsAg loss is a major goal of current treatment efforts in CHB.11 In HBeAg-negative CHB patients, the goal of antiviral therapy is a sustained off-treatment response and HBsAg clearance.49 However, because it is difficult to obtain HBsAg clearance during NA therapy, the reduction of HBsAg and HBcrAg might be useful when judging the efficacy of NA therapy. Therefore, HBcrAg might be more useful in the HBeAg-negative phase. With reference to the first published papers,53,54 the Japan Society of Hepatology (JSH) Guidelines for the Management of HBV Infection53 described the criteria for the cessation of NA therapy (see Table 15).13 The three laboratory criteria for the cessation of NA therapy are: 1) at least 2 years administration of NAs; 2) undetectable serum HBV DNA levels (using real-time polymerase chain reaction); and 3) negative serum HBeAg at time of treatment cessation. When these criteria are met, the risk of relapse can be determined from HBsAg and HBcrAg levels at the time of cessation of therapy.

A decrease in HBcrAg was observed under NA therapy, and the pattern of reduction may provide predictive information on the risk of post-treatment HBV reactivation.55 A serum HBcrAg level >3.7 log U/mL at NA cessation predicted virological relapse within 1 year of NA cessation.56 Therefore, serum HBcrAg as a surrogate marker, may have a better influence on patients who are planning NA cessation.

Regarding entecavir (ETV) or tenofovir (TDF), the HBcrAg level at NA cessation is an independent relapse predictor, as well as HBsAg, age, alanine aminotransferase (ALT), and TDF use. Hsu et al.57 enrolled 135 CHB patients who had stopped ETV or TDF after accomplishing viral reduction for a median of 25.2 months. All patients stopped NA with negative HBeAg and undetectable HBV DNA. During the follow-up period (median, 25.9 months), clinical relapse and HBsAg loss occurred in 66 and eight patients, respectively, with a 5-year cumulative incidence of 56.1% and 8.8%, respectively. A SCALE-B score was calculated using the equation...
35 × HBsAg (log IU/mL) + 20 × HBcAg (log U/mL) + 2 × age (years) + ALT (U/L) + 40 for TDF use. The concordance rates for clinical recurrence were 0.87, 0.88, 0.87, 0.85, and 0.90 at 1, 2, 3, 4, and 5 years, respectively. Moreover, complete HBsAg loss occurred in low-risk patients predicted by the score. 57

HBcAg in the prediction of HCC occurrence and recurrence

To predict which patients will develop advanced liver disease including HCC during NA treatment is difficult. 58 High levels of HBV DNA were correlated with increased risk of cirrhosis and HCC development. 59 Low or undetectable HBV viral load decreased the risk of HCC development but did not prevent it completely. 59-61 Several HBV markers are related to HCC development in CHB patients including serum HBcAg level (Table 1). 62-64

HBcAg was superior to HBV DNA in terms of predictive power for HCC development in treatment-naïve CHB patients. 64 During follow-up (median, 10.7 years), 78 of 1,031 (7.6%) CHB patients without NA treatment developed HCC. HBcAg >2.9 log U/mL (hazard ratio [HR], 5.05; 95% CI, 2.40–10.63) and basal core promoter (BCP) mutations (HR, 28.85; 95% CI, 4.00–208.20) were independently associated with HCC occurrence. 62-64 Moreover, complete HBsAg loss occurred in low-risk patients predicted by the score. 57

A recent report showed that a combination of HBsAg and HBcAg values was an effective biomarker for evaluating HCC occurrence in CHB patients. 67 When cut-off values of HBsAg and HBcAg were defined as 3.0 log U/mL and 3.0 log U/mL, respectively, patients with HCC history were frequently found in the low HBsAg (P=0.002) and high HBcAg (P<0.001) groups. When HBsAg and HBcAg were combined, HCC history was most frequent in the subset with low HBsAg and high HBcAg, among HBcAg-negative patients, despite NA therapy.

HBcAg level before surgery might be a potential marker to stratify post-surgical surveillance approaches and to identify patients with a high risk of HCC recurrence. A recent study also confirmed the predictive value of HBcAg for HCC recurrence after curative surgery. In a study of 55 patients, a serum HBcAg level >4.8 log U/mL at the time of HCC diagnosis gave an HR of 8.96 for subsequent HCC recurrence within 2 years. 67 Finally, the HCC recurrence-free survival rates were significantly lower in HCC patients with high intrahepatic cccDNA and serum HBcAg levels than those with low cccDNA/HBcAg levels (P=0.035 and P=0.003, respectively). 68

LIVER FIBROSIS MARKER: M2BPGi

Innovative glycoproteomic studies revealed that fibrosis leads to a specific modification of the glycosylation and sugar chain structure on the Mac-2 binding protein (M2BP). 69,70 Based on these observations, M2BPGi has been developed as a novel surrogate serum glycoprotein-based biomarker (glycobiomarker) that reflects fibrosis stage. Here, we describe the improvement and latest assessment of M2BPGi, as a surrogate marker of HBV-related liver fibrosis and as a predictor of HBV-related HCC occurrence.

M2BPGi and its features

In 2013, M2BPGi (also called Wisteria floribunda agglutinin [WFA]-positive M2BP [WFA⁺·M2BP]) was introduced as a novel, noninvasive, rapidly assayed serological glycobiomarker for the evaluation of liver fibrosis. 69 Fibrosis results in specific modification of the glycosylation and sugar chain structure of M2BP, whereby levels of these modified M2BP proteins correlate significantly with the progression of fibrosis. 69,70

Quantification assays of serum M2BP with fibrosis-specific modified sugar chain structures, i.e., M2BPGi, were developed, evaluated, and approved for clinical use in Japan. 69-71 M2BPGi is...
detected using a specific lectin called WFA that recognizes the N-acetylgalactosamine residue of N-glycans and O-glycans on M2BP (Fig. 3A). An automated and high-throughput assay measures M2BPGi levels in 10 µL serum samples in 17 minutes (Fig. 3B). The M2BPGi assay (Sysmex Corp., Hyogo, Japan) has a reported cut-off index (C.O.I.) range of 0.1–20 and samples with less than 1.0 C.O.I. are considered negative.

M2BPGi for HCC prediction in CHB

M2BPGi is predominantly used to evaluate liver fibrosis in CHC patients. However, the diagnostic value of M2BPGi to assess liver fibrosis was demonstrated in patients with viral hepatitis, CHB, non-alcoholic fatty liver disease, and biliary atresia, as well as CHC. In addition, M2BPGi levels are prognostic of the risk of HCC development in CHB, CHC, or non-alcoholic fatty liver disease. Furthermore, post-sustained virological response M2BPGi is a predictive factor for the development of HCC in patients with no previous HCC history and those treated with DAAs for hepatitis C virus (HCV) infection. In this section, we summarize studies showing M2BPGi has good performance for the assessment of liver fibrosis and HCC prediction in CHB.

Kim et al. reported that M2BPGi level was an independent predictor of HCC development (adjusted HR, 1.143; 95% CI, 1.139–1.829), together with male gender and diabetes (all P<0.05) in multivariate analysis. In patients without cirrhosis (n=1,087), M2BPGi levels ≥1.8 were associated with a higher risk of HCC development (P<0.001), whereas M2BPGi levels ≥1.8 tended to be associated with a higher risk of HCC development in patients with cirrhosis (n=236) (P=0.073).

Conversely, M2BPGi accurately identified CHB patients with a low risk of HCC. Mak et al. evaluated M2BPGi for HCC prediction in 207 non-treated HBeAg-negative patients with recognized HBeAg seroconversion. Using a cut-off of 0.68 C.O.I., the baseline M2BPGi showed good performance (area under the curve [AUC], 0.88; sensitivity, 90%; specificity, 80%). In these HBeAg-negative patients, the NPV was very high (99.3%), while the positive predictive value was low (25.8%).

Additionally, M2BPGi was more effective than AFP for predicting HCC occurrence and was an independent predictor of HCC. Jun et al. enrolled 947 treatment-naive patients mono-infected with HBV or HCV and without HCC at baseline. The median M2BPGi was significantly higher among patients with cirrhosis (2.67 vs. 0.80; P<0.001) and those who developed HCC (3.22 vs. 1.16; P<0.001). M2BPGi outperformed AFP for patients with CHB (0.84

![Figure 3. Detection of WFA-M2BP](https://example.com/image1.png)

**Figure 3.** Detection of WFA-M2BP. (A) Structure of WFA-M2BP. WFA-M2BP, which was recently shown to be a liver fibrosis glyco-biomarker with a unique fibrosis-related glycoalteration, has multibranching and sialylated N-glycans. WFA lectin binds specifically to the glycosylation isomer. (B) Quantification of serum WFA-M2BP. WFA-M2BP quantification is measured based on a lectin-antibody sandwich immunoassay using a fully-automatic immunoanalyzer HISCL-2000i (Sysmex Corp., Hyogo, Japan). The lectin-binding WFA-M2BP recognizes ALP-labelled antibody in a chemiluminescence enzyme immunoassay (CLEIA). Every reaction is accustomed to the platform during the automatic assay, which is finished after 17 minutes. M2BP, Mac-2 binding protein; M2BPGi, Mac-2-binding protein glycosylation isomer; ALP, alkaline phosphatase; WFA-M2BP, Wisteria floribunda agglutinin-positive Mac-2 binding protein.
In CHB patients receiving NA treatment, M2BPGi level before treatment and 48 weeks after the start of NA treatment is an indicator of HCC occurrence. Hsu et al. reported the baseline level can be factored into the risk prediction of HCC in NA-treated cirrhosis patients. Baseline M2BPGi level was associated with HCC risk (HR, 1.07 per C.O.I.; 95% CI, 1.01–1.14) in cirrhosis patients, whereas year 1 or 2 levels were not independently predictive. Shinkai et al. reported serum M2BPGi levels ≥1.215 C.O.I. at 48 weeks were associated with HCC development (HR, 5.73; \( P \leq 0.001 \)).

In patients with undetectable HBV DNA during NA therapy, higher pre-treatment M2BPGi level was associated with increased risk of HCC development. Cheung et al. compared 57 NA-treated patients with undetectable HBV DNA and HCC development with 57 controls. There was a significant difference in median levels of pre-treatment M2BPGi between HCC and control groups (0.67 vs. 0.41 C.O.I.; \( P < 0.001 \)). Among patients with cirrhosis, the median level of M2BPGi was higher in the HCC group than the control group (0.74 vs. 0.47 C.O.I.; \( P = 0.014 \)). Among patients without cirrhosis, the median level of M2BPGi of the HCC group was also higher (0.48 vs. 0.28 C.O.I.; \( P = 0.002 \)). With a cut-off value of 0.69 C.O.I., the AUROC of pre-treatment M2BPGi to predict HCC development for the whole cohort was 0.70. With cut-off values of 0.69 and 0.34 C.O.I. for patients with and without cirrhosis, AUROCs that predicted HCC were 0.67 and 0.77, respectively.

In patients with HBV-related HCC, Kim et al. showed that the level of M2BPGi is an independent predictive factor of HBV-related HCC recurrence after curative resection. Also, Heo et al. evaluated its accuracy in assessing liver fibrosis and in predicting the risk of developing HCC in patients with CHB. They concluded the level of M2BPGi significantly reflected degree/extent of liver fibrosis and independently predicted the risk of developing HCC in patients with CHB.

Therefore, M2BPGi might be suitable for assessing fibrosis level and prediction of HCC in CHB patients. Clinical applications of M2BPGi in patients with chronic liver disease are summarized in Table 2.

### TUMOR MARKERS: AFP, AFP-L3, PIVKA-II, DKK-1 AND CIRCULATING IgG ANTIBODIES

Measuring serum levels of tumor biomarkers for HCC is essential for CHB management. AFP, AFP-L3, and PIVKA-II are HCC-specific tumor markers. Although tumor marker levels are not included in diagnostic criteria for HCC, screening recommendations in the guidelines of the American Association for the Study of Liver Diseases, or the European Association for the Study of the Liver, provide valuable supportive information for diagnosing HCC.

Levels of AFP, AFP-L3, and PIVKA-II usually increase as HCC develops, i.e., with increased size and number of HCC lesions and progression to portal vein invasion. Other studies reported increased tumor marker levels suggested a high degree of HCC malignancy regardless of morphological progression.

### Table 2. Clinical applications of M2BPGi in CHB patients

<table>
<thead>
<tr>
<th>Category</th>
<th>Finding</th>
<th>M2BPGi level (C.O.I.) and point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver fibrosis</td>
<td>Significant fibrosis (≥F2) in CHB</td>
<td>≥1.06 C.O.I. (AUC, 0.753)</td>
<td>73</td>
</tr>
<tr>
<td>HCC occurrence/recurrence</td>
<td>Higher risk of HCC development in CHB patients</td>
<td>≥1.8 C.O.I. for patients without cirrhosis (( P &lt; 0.001 ))</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Low risk of HCC in HBeAg-negative patients</td>
<td>≤0.68 C.O.I. at baseline</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Risk for HCC development in CHB patients with cirrhosis treated with NAs</td>
<td>M2BPGi-based score* ≥652.5 at baseline</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Risk for HCC development in CHB patients treated with NAs</td>
<td>≥1.215 C.O.I. at 48 weeks</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>HCC development in CHB patients</td>
<td>≥0.69 C.O.I. at baseline</td>
<td>85</td>
</tr>
</tbody>
</table>

M2BPGi, Mac-2 binding protein glycosylation isomer; CHB, chronic hepatitis B; C.O.I., cut-off index; AUC, area under the curve; HCC, hepatocellular carcinoma; HBeAg, hepatitis B envelope antigen; NAs, nucleos(t)ide analogues.

*M2BPGi-based score, 8 × age (years) + 7 × baseline M2BPGi (COI) + 10 × body mass index (kg/m²). The score was calculable in 171 CHB patients with cirrhosis with a median of 652.5 (IQR, 581.3, 709.4) point.
prognosis after initial diagnosis. An increased level of DKK-1 occurs in a wide variety of cancers, including multiple myeloma, prostate cancer and HCC. Circulating anti-CD25 IgG antibodies may have prognostic rather than early diagnostic values for HCC. Circulating IgG antibody to p16 protein might be a useful biomarker for HCC prognosis assessment rather than for early diagnosis of HCC.

In this section, we introduce three tumor markers, AFP, AFP-L3, and PIVKA-II, as indicators of HCC development and predictors of patient outcome. In addition, we provide information of DKK-1 and circulating IgG antibodies as novel markers of HCC.

**AFP and AFP-L3**

AFP was first discovered in the serum of HCC patients in 1964 and has since been the primary diagnostic biomarker for HCC. It is normally produced at a low concentration by liver, yolk sac, and gastrointestinal tract during fetal and neonatal development. High levels of serum AFP correlated with the presence and development of HCC and it is used as a diagnostic and prognostic factor.

Despite this, there are multiple problems using AFP as a diagnostic marker, such as elevation in non-HCC diseases including cirrhosis, hepatitis, cholangiocarcinoma, testicular germ cell tumor, and metastatic colon cancer. Because of its low diagnostic accuracy, with sensitivities ranging from 18–60% and specificity of 85–90%, AFP has recently been excluded from American Association for Study of Liver Diseases (AASLD) HCC surveillance guidelines. Indeed, only 60–80% of HCC have elevated AFP levels, leaving a large margin for false-negatives and missed diagnoses. Therefore, AFP alone is not recommended as the main screening test for HCC. To significantly improve the diagnostic accuracy of HCC, additional biomarkers are needed to complement AFP.

AFP exists as three glycoforms, each with different binding competence to lectin Lens culinaris agglutinin. AFP-L1 is a non-binding fraction, AFP-L2 is a weak binding fraction, and AFP-L3 is a binding fraction. AFP-L1 is increased in chronic hepatitis and liver cirrhosis, whereas AFP-L3 is specifically increased in HCC. Because AFP-L3 has a high specificity of 92.0–99.4% for HCC and a low sensitivity of 18.8–37.0%, it is considered a more specific biomarker for HCC. Moreover, because AFP-L3 is typically not detected when AFP levels are <20 ng/mL, AFP-L3 is not relevant for the diagnosis of HCC in individuals with a total AFP concentration <20 ng/mL. Thus, the sensitivity for AFP-L3 appears to be adversely affected by the total AFP concentration. Furthermore, AFP and AFP-L3 levels were detected in blood 6 months prior to a diagnosis of HCC.

Recent improvements in highly sensitive analytical methods have enhanced the sensitivity of the AFP-L3 immunoassay, referred to as “highly sensitive AFP-L3” (hs-AFP-L3). Toyoda et al. examined patients with benign liver disease (n=44) including chronic hepatitis, cirrhosis, and HCC (n=54) and determined the performance of hs-AFP-L3. The sensitivity and specificity of hs-AFP-L3 were 84.9% and 88.6%, respectively. Additionally, postoperative AFP-L3% was a prognostic factor of HCC recurrence after curative treatment and detected small tumors and early stage HCC. These results indicate hs-AFP-L3% might be a valuable biomarker for detecting early-stage HCC.

**PIVKA-II**

PIVKA-II, known as des-γ-carboxyprothrombin, is an abnormal prothrombin molecule that is increased in HCC. During malignant transformation in hepatocytes, the vitamin K-dependent carboxylase system becomes damaged; a deficiency in post-translational carboxylation leads to the production of PIVKA-II. During this process, PIVKA-II loses its normal prothrombin function but may promote malignant proliferation in HCC. One benefit of PIVKA-II is that it is less likely to be elevated in non-HCC liver diseases than AFP. In a screening test for HCC, when compared to cases of cirrhosis and chronic hepatitis, PIVKA-II yielded a sensitivity of 72.7% and a specificity of 90.0%, which was comparable to AFP.

Recently, the performance of AFP was compared with PIVKA-II for the diagnosis of early-stage HCC in France. This study included 43 cirrhosis patients and 85 HCC patients including 32 patients with early HCC. PIVKA-II (threshold value, 42 mAU/mL) performed better than AFP (threshold value, 5.5 ng/mL) for early-stage HCC diagnosis (AUC, 0.81; 95% CI, 0.697–0.924 vs. AUC, 0.582; 95% CI, 0.443–0.722), and a PIVKA-II level >90 mAU/mL was an independent predictor of microvascular invasion (HR, 3.5; 95% CI, 1.08–11.8; P=0.043), a major prognostic factor in HCC.

As described above, PIVKA-II is a potential serum biomarker for the early diagnosis of HCC. Furthermore, Chen et al. reported a meta-analysis where PIVKA-II had a better accuracy than AFP for the detection of HCC in CHB, regardless of tumor size, patient ethnic background (American, European, Asian, or African), or etiology of HCC (HBV-related or mixed).

Most importantly, serum PIVKA-II level is a suitable parameter
for the development of portal venous invasion.114 Koike et al.114 enrolled 227 HCC patients who did not show portal venous invasion and who received percutaneous ethanol injection therapy and/or microwave coagulation therapy at the time of their first hospital admission. After their HCC was treated, the patients were followed for a mean of 19 months. Of the 227 patients, 24 (11%) later developed portal venous invasion, and the PIVKA-II level at the time of initial diagnosis of HCC had a significant correlation with the later development of portal venous invasion.114 Yamashita et al.115 reported that even in cases with small HCC (≤2 cm), patients with a high PIVKA-II level (>100 mAU/mL) were at risk for microinvasion. Therefore, in such patients, hepatic resection with a wide tumor margin should be recommended.115

Moreover, Lee et al.116 investigated whether pre- or post-ablation serum AFP and PIVKA-II levels predicted prognosis in patients with curative radiofrequency ablation (RFA) for HBV-related HCC. They retrospectively analyzed 412 patients with HBV-related single HCC treated with percutaneous RFA. AFP and PIVKA-II levels were measured before and 1 month after treatment. Among the tumor markers, post-ablation PIVKA-II was an independent prognostic factor for overall and recurrence-free survival (HR, 3.438; 95% CI, 1.331–8.877; \( P = 0.011 \) and HR, 4.934; 95% CI, 2.761–8.816; \( P < 0.001 \), respectively). They concluded that post-ablation serum PIVKA-II is a useful biomarker for predicting survival and recurrence after curative RFA in patients with HBV-related HCC.116

**Combination of AFP and PIVKA-II**

Because PIVKA-II is more specific to HCC and has a reduced tendency to be elevated in other chronic liver diseases, a combination of AFP and PIVKA-II had a higher efficiency for the diagnosis of early HCC in cirrhosis patients. Lok et al.117 reported a combination of AFP and PIVKA-II had increased sensitivity (65% to 87%) compared with AFP alone, but decreased specificity (84% to 69%). For screening, this increased sensitivity is obviously valuable.117 Song et al. reported that in 120 patients with HCC, PIVKA-II alone was inferior to the combination of AFP and PIVKA-II because of its lower sensitivity (53.3% vs. 78.3%).118 A recent meta-analysis including 11 studies by Caviglia et al.119 also described that the use of AFP and PIVKA-II may improve the effectiveness of surveillance among patients at risk for HCC development. The weighted summary AUC (sAUC) of PIVKA-II and AFP for the discrimination between patients with HCC and those without was 0.791 (0.746–0.837) and 0.767 (0.732–0.803), respectively. The combination of PIVKA-II and AFP resulted in a sAUC of 0.859 (0.837–0.882), suggesting that the performance for HCC detection of PIVKA-II and AFP was significantly superior to each biomarker used alone (ΔsAUC, 0.068; \( P = 0.032 \) and ΔsAUC, 0.092; \( P < 0.001 \), respectively).119

Previous studies suggested specific biomarkers for HCC diagnosis, of which the combination of AFP and PIVKA-II appeared to be the best. Chen et al.120 selected five representative biomarkers (AFP, AFP-L3, PIVKA-II, squamous cell carcinoma antigen, and centromere protein F autoantibody) with diagnostic potential for detecting HCC. Serum levels of the five biomarkers were simultaneously measured in a large sample set (n=846) including patients with HCC, cirrhosis, chronic HBV infection, and healthy controls.120 Overall, for two-marker combinations, a prediction algorithm including AFP and PIVKA-II had the best diagnostic value. The apparent AUC (without correction) of the two-marker algorithm in detecting early-stage HCC was 0.86 (95% CI, 0.81–0.90). Moreover, in another report of the diagnosis of HCC associated with alcoholic and non-alcoholic fatty liver disease, AFP and PIVKA-II appeared to be the best combination of biomarkers,121 suggesting that PIVKA-II might be a complementary biomarker for the diagnosis of HCC and to improve the identification of patients with AFP-negative HCC. Meanwhile, as the recent report, combined microRNA-122 (miR-122), AFP plus PIVKA-II had an adjusted HR for HCC development of 10.63.122 AUCs were 0.675 for miR-122, 0.791 for AFP and 0.846 for PIVKA-II, respectively, while their combination improved the discrimination power between cirrhosis and HCC (AUC, 0.918).122

**DKK-1**

DKK-1 is a glycoprotein which is a secretory antagonist of the Wnt/beta-catenin signaling pathway.123 Although its detailed function is not completely understood, the increase of DKK-1 expression occurs in a wide variety of cancers, including multiple myeloma, prostate cancer and HCC.124 Several studies have shown that higher DKK-1 levels in HCC patients than in healthy individuals,95,124–126 or than in patients with cirrhosis without HCC.126–128 Shen et al.126 reported serum DKK-1 and AFP levels in a cohort of 1,284 patients. DKK-1 was more useful for detection of AFP-negative HCC, while combining DKK-1 with AFP enhanced the detection rate of early-stage HCC to help in detection in HCCs, which do not produce AFP.126 Another report from South Korea showed that the combination of AFP and DKK-1 was just slightly better than AFP alone for the detection of early-
stage HCC (AUC, 0.693 vs. 0.691) in HCC patients with mainly HBV-infection (n=208).

In addition, a meta-analysis published in 2014 containing four studies and other recently published study showed that higher levels of DKK-1 expression in patients with HCC were associated with the lower survival rate.

Circulating IgG antibodies

In recent studies, Wang et al. found that serum IgG antibodies against linear peptide antigens derived from p16 protein, interleukin 2 receptor α-subunit (also called CD25) and forkhead/winged-helix transcription factor box P3 (FOXP3) were significantly changed in the patients with HCC. Therefore, circulating IgG antibodies for these target molecules may be either diagnostic or prognostic values for solid tumors. Serum levels of IgG antibodies for p16α, CD25α and FOXP3 were significantly higher in HCC patients than control subjects.

Further analysis showed that increased levels of plasma IgG for these three peptide antigens were mainly shown in patients with the intermediate and late-stage HCC. This study has confirmed that serum IgG antibodies to p16, CD25 and FOXP3 are significantly increased in HCC patients, especially in late-stage HCC. These autoantibodies may be useful biomarkers for assessment of HCC prognosis.

DISCUSSION

In this review, we described the characteristics and clinical applications of novel biomarkers. HBcrAg is appropriate for evaluating the amount of intrahepatic cccDNA in CHB patients. M2BPGi can assess the severity of liver fibrosis and the risk of HCC development in CHB patients. Regarding tumor markers including AFP, PIVKA-II, and AFP-L3, DKK-1 and circulating IgG antibodies, using only one had a restricted detection rate for HCC, but the combination by two showed improved detection rates.

Here, we discuss the future prospects of HBcrAg, M2BPGi, and tumor markers. First, the amount of HBcrAg is associated with that of HBV DNA in all CHB disease states. Even in patients who achieve a “functional cure” with undetectable serum HBV DNA and HBsAg, severe complications including HBV reactivation and HCC occurrence may be reported. Because some patients achieving a “functional cure” still have detectable serum HBcrAg, prospective studies comparing the long-term outcome between HBcrAg-positive and HBcrAg-negative patients are required. To date, most reports have been published from East Asia. For HBcrAg to be used more in clinical practice, large cohort studies should be completed in other areas, especially the United States and Europe.

To confirm a functional cure of HBV, higher sensitivity assays for HBsAg and HBcrAg are required. Recently, a new HBcrAg assay with approximately 10-fold higher sensitivity is being developed. Furthermore, based on a fully-automated pretreatment technique before HBcrAg measurement, new highly sensitive HBcrAg measurements should be used to monitor HBeAg-negative patients.

Second, M2BPGi has been used as a valuable biomarker to evaluate liver fibrosis, especially in CHC patients. Although past studies have documented the accuracy of M2BPGi assay in patients with CHC, few have addressed CHB. In recent studies, Wang et al. found that serum IgG antibodies to p16a, CD25a and FOXP3 were significantly higher in CHB patients than control subjects.

Further analysis showed that increased levels of plasma IgG for these three peptide antigens were mainly shown in patients with the intermediate and late-stage HCC. This study has confirmed that serum IgG antibodies to p16, CD25 and FOXP3 are significantly increased in HCC patients, especially in late-stage HCC. These autoantibodies may be useful biomarkers for assessment of HCC prognosis.

The M2BPGi cut-off value for predicting cirrhosis or HCC varies, depending on disease etiology. Ichikawa et al. reported that M2BPGi ≥0.71 was a risk factor for HCC development in CHB patients. As described above, M2BPGi levels ≥1.215 48 weeks after starting NA treatment were associated with HCC. Taken together, the M2BPGi levels of CHB patients seem to be lower than those of CHC patients. Conversely, in CHC independent of liver fibrosis, whether the M2BPGi level is increased is still controversial. Only one study has evaluated M2BPGi as a predictor of HCC in non-Asian patients and patients outside East Asia. Further studies should evaluate M2BPGi as an HCC biomarker in broader patient populations, including non-Asians and those with severe liver fibrosis.

Third, although the diagnosis of HCC remains difficult, especially in the early stage, the early and accurate diagnosis of HCC is vital to improve CHB patient outcomes. Current information suggests no single biomarker is likely to have optimal sensitivity and specificity for the detection of HCC, particularly at the early stages of development. Many studies reported combinations of biomarkers improved the detection of early stage HCC. Additional studies are needed to evaluate further the effectiveness of combined biomarkers for HCC diagnosis.

Recent studies reported a combination of these three tumor markers had very high sensitivity and specificity for diagnosing HCC without the use of imaging studies, and improved the sensitivity and specificity of early HCC diagnosis. In the JSH-HCC guidelines, simultaneous measurement of AFP, AFP-L3, and PIVKA-II with ultrasound examination is recommended for HCC surveillance of high-risk populations.

When considering HCC biomarker applications, there must be a careful and an accurate consideration as to the various metabolic
profiles of variable ethnic groups.\textsuperscript{137} Nguyen et al.\textsuperscript{138} reported clear ethnic differences in the diagnostic value of AFP. Patients with HCV-related HCC in Asia, Europe, and Central and South America had a normal AFP level of 18\%, whereas African-American patients had normal level of 43\%. Furthermore, there was difference between the underlying etiologies of liver disease, where HCV-related HCC was more strongly associated with elevated AFP compared with HBV-related HCC.\textsuperscript{138} Further studies are needed to evaluate PIVKA-II as an HCC biomarker in broader patient populations, including non-Asians and those with mild liver fibrosis. In summary, etiological, dietary, genetic, and environmental factors that differ between populations suggest the need for validation studies in various regions to establish better diagnostic and screening tools.

**CONCLUSIONS**

HBcrAg and M2BPGi are useful novel biomarkers for the management of CHB including predicting HCC occurrence. AFP, AFP-L3, PIVKA-II, DKK-1 and circulating IgG antibodies are HCC-specific tumor markers. Combinations of these biomarkers might have better prospects for application in clinical practice. Further global studies are required to increase the application of these useful biomarkers for many aspects of CHB clinical practice.

**Authors’ contributions**

Conceptualization, T.I. and Y.T.; Writing and Original Draft Preparation, T.I.; Writing, Review and Editing, Y.T.

**Acknowledgements**

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**Conflicts of Interest**

Takako Inoue is currently supported by a research grant from Gilead Sciences and MSD.K.K. Yasuhito Tanaka is currently conducting research sponsored by Chugai Pharmaceutical Co., Ltd., Bristol-Myers Squibb, Fujirebio, Inc., and Gilead Sciences. Lecture fees are follows: Fujirebio, Inc. and Gilead Sciences.

**REFERENCES**


32. Hadziyiannis E, Laras A. Viral biomarkers in chronic HBeAg negative hepatitis B infection. Genes (Basel) 2018;9:469.


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


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Novel biomarkers in management of CHB
Role of cytoglobin, a novel radical scavenger, in stellate cell activation and hepatic fibrosis

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Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, Japan

Cytoglobin (Cygb), a stellate cell-specific globin, has recently drawn attention due to its association with liver fibrosis. In the livers of both humans and rodents, Cygb is expressed only in stellate cells and can be utilized as a marker to distinguish stellate cells from hepatic fibroblast-derived myofibroblasts. Loss of Cygb accelerates liver fibrosis and cancer development in mouse models of chronic liver injury including diethylnitrosamine-induced hepatocellular carcinoma, bile duct ligation-induced cholestasis, thioacetamide-induced hepatic fibrosis, and choline-deficient L-amino acid-defined diet-induced non-alcoholic steatohepatitis. This review focuses on the history of research into the role of reactive oxygen species and nitrogen species in liver fibrosis and discusses the current perception of Cygb as a novel radical scavenger with an emphasis on its role in hepatic stellate cell activation and fibrosis. (Clin Mol Hepatol 2020;26:280-293)

Keywords: Cytoglobin; Hepatic stellate cells; Fibrosis; Free radical scavengers; Antioxidants

INTRODUCTION

Liver fibrosis is a wound-healing response to chronic liver diseases (CLDs) and often results in cirrhosis, liver failure, portal hypertension, and hepatocellular carcinoma (HCC). CLD has various etiologies including alcohol consumption, infectious diseases such as viral hepatitis, exposure to toxins and drugs, persistent autoimmune injury, or chronic conditions of altered metabolism. Whatever its etiology, liver fibrogenesis is a dynamic and highly integrated molecular, tissue, and cellular process that distorts the hepatic architecture, and contributes to the formation of a new biochemical environment in the liver.

The relevance of reactive oxygen species (ROS) to liver fibrosis was first described in 1965 by Comporti et al. and Ghoshal and Recknagel who reported that liver injury was induced by carbon tetrachloride (CCl4) via lipid peroxidation. Two years later, Di Luzio and Hartman implicated lipid peroxidation in the pathogenesis of ethanol-induced fatty liver. Figure 1 shows the timeline of research on the role of oxidative stress in liver diseases. Almost 50 years have passed, and today oxidative stress is known to be
Cytoglobin suppresses HSC activation and fibrosis

Le Thi Thanh Thuy, et al.

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related to chronic liver injury and hepatic fibrosis caused by ethanol, CCl₄, non-obese patients with NASH, iron overload, and hepatitis C virus (HCV). Moreover, mitochondrial ROS mediate metabolic pathway signaling, and their changes affect the development and progression of CLD. The fibrogenic progression of these diseases is associated with a significant decrease in, and/or impairment of, antioxidant defenses such as superoxide dismutase (SOD) 2 activity, or manganese (Mn) SOD.

The recently discovered cytoglobin (Cygb), which has an antioxidant function, is present in hepatic stellate cells (HSCs), the main cell type involved in liver fibrosis. In proteomic analyses, Kawada et al. has found this Cygb protein in rat HSCs and named it as stellate cell activation-associated protein. Later, it was classified as the fourth member of the globin family in mammals, after hemoglobin (Hb), myoglobin (Mb), and neuroglobin (Ngb), and renamed as Cygb. Figure 2 showed the 3D structure of these four members of globin family in human. Cygb is a 21-kDa protein consisting of 190 amino acids that shows ~25% identity with vertebrate Mb and Hb, and 16% identity with human Ngb. Moreover, some key residues in the ligand-binding reaction are highly conserved among different species of Ngb, Mb, and Hb. By contrast, both Cygb and Ngb have unusual features, which are different from traditional pentacoordinated globins such as Mb and Hb. Spectroscopic studies have shown that Cygb and Ngb contain a hexacoordinated heme iron, to which two his imidazole groups are bound directly in both the deoxyferrous and ferric states. Therefore, exogenous ligands, such as O₂ or carbon monoxide, can bind to the iron after displacement of one of his imidazole groups from the axial coordination site. However, like Mb, Cygb exhibits high intrinsic affinity for O₂.

<table>
<thead>
<tr>
<th>Year</th>
<th>Landmark in ROS research in liver diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>Role of ROS on liver injury induced by CCl₄ throughout lipid peroxidation</td>
</tr>
<tr>
<td>1967</td>
<td>Mechanism of free-radical-induced liver tissue injury</td>
</tr>
<tr>
<td>1984</td>
<td>Formation of free-radical intermediates by rat liver microsomes incubated with ethanol, 2-propanol, or 2-butanol</td>
</tr>
<tr>
<td>1985</td>
<td>Ethanol triggered ROS formation</td>
</tr>
<tr>
<td>1987</td>
<td>Lipid peroxidation linked to collagen production in hepatic injury</td>
</tr>
<tr>
<td>1990s</td>
<td>Oxidative stress in iron overload and HepC</td>
</tr>
<tr>
<td>2000s</td>
<td>Alter oxidative metabolism of triglyceride at both hepatic and extra-hepatic levels increased oxidative stress in NASH</td>
</tr>
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</table>

**Figure 1.** Timeline of research into ROS in liver disease. ROS, reactive oxygen species; CCl₄, carbon tetrachloride; NASH, non-alcoholic steatohepatitis.

**Figure 2.** 3D structure of four members of globin family. Human haemoglobin (RCSB Protein Data Bank accession number: 2HHB, tetramer), cytoglobin (1V5H, dimer), neuroglobin (1OJ6, monomer), and myoglobin (3RGK, monomer) are shown with alpha-helices and heme group. Color scheme for the elements: grey for carbon, red for oxygen, blue nitrogen, yellow for sulfur.

disulfide bridges.\textsuperscript{42,43} Their substitution or reduction diminish Cygb affinity to O\textsubscript{2}. This indicates that the cellular redox state influences protein structure by S-S bond formation or cleavage, thus affecting O\textsubscript{2} binding.

Not only structural but also functional and pathophysiological characterizations of Cygb have been reported. This review highlights the protective role of Cygb in liver fibrosis via its ROS-scavenging function and discusses its future prospects.

**CYGB: TISSUE AND CELLULAR DISTRIBUTION**

Cygb is expressed ubiquitously in all vertebrate organs, including the brain, liver, heart, lung, retina, gut, and esophagus. The expression of Cygb in mouse organs was shown in Figure 3. Looking at the liver, Cygb is detected in HSCs,\textsuperscript{36} but not in hepatocytes, Kupffer cells (KCs), endothelial cells, or myofibroblasts.\textsuperscript{37} It is also present in the stromal cells of the red pulp of the spleen. In the kidney, Cygb expression is present in stromal cells along the proximal and distal uriniferous tubules.\textsuperscript{44} It is expressed in fibroblasts, but not in cardiomyocytes in the heart. In the lung, it is found in chondroblasts or stromal cells along alveolar walls. In the thymus, it is present in stromal cells. Interestingly, Cygb is also found in adipose tissue (Fig. 3). A novel expression site is in melanocytes; its absence is associated with the melanocyte-to-melanoma transition.\textsuperscript{45}

At the cellular level, Cygb is mainly expressed in fibroblast-related cell lineages such as HSCs, chondroblasts, and osteoblasts.\textsuperscript{37,46-52} Moreover, it has been found in distinct neuron populations.\textsuperscript{47,50,53} Cytoplasmic and nuclear Cygb localization mainly occurs in neurons. Specific Cygb expression in neurons suggests that the globin may play different roles in this cell population compared to mesenchymal cells.\textsuperscript{57} Some studies have demonstrated that Cygb localizes in macrophages, muscles,\textsuperscript{54} hepatocytes,\textsuperscript{53} and epithelium.\textsuperscript{53-55} However, the discrepancies between the studies on the cell-type and subcellular localization of Cygb might have arisen due to technical issues related to the specificity of the antibodies used, immunodetection methods applied, and endogenous Cygb expression level.\textsuperscript{56}

**CYGB: A NOVEL RADICAL SCAVENGER**

**Impact of radicals in liver fibrosis**

Oxygen free radicals, more generally known as ROS, along with reactive nitrogen species (RNS) are the most important radical species generated in living systems.\textsuperscript{57} ROS and RNS play an important role in the establishment of fibrosis and subsequently in cirrhosis.\textsuperscript{58} In a previous study, human HSCs cultured with human neutrophils stimulated to produce ROS showed evidence of not only oxidative stress but also increased procollagen α1(I) mRNA and protein levels compared to those co-cultured with unstimulated neutrophils.\textsuperscript{59} These effects were inhibited by adding vitamin E or SOD to the culture. By contrast, the intra- and extracellular hydrogen peroxide, lipid peroxidation, and collagen type I levels

![Figure 3](https://example.com/figure3.png)  
*Figure 3.* Cygb expression in multiple organs of mice. Immunohistochemical staining of Cygb antibody (our laboratory\textsuperscript{103}) showing dense Cygb positive cells in all organs. Representative red arrows, Cygb positive cells. Original magnification, ×400. Cygb, cytoglobin.
were increased in HSCs co-cultured with HepG2 cells, which express cytochrome P450 2E1 (CYP2E1). In another study, an increase in collagen production was prevented by antioxidants and a CYP2E1 inhibitor. Similarly, co-culture with KCs results in activation and proliferation of HSCs, along with increased production of collagen type I and hydrogen peroxide. Thus, induction of oxidative stress in inflammatory cells, macrophages, and CYP2E1-positive hepatoma cells leads to the production of pro-fibrogenic mediators. In patients with alcoholic liver disease and NASH, CYP2E1 plays a critical role in ROS generation and nitrotyrosine which in turn cause nucleotide modifications in DNA and induce dysfunction and degradation of several functional proteins. In a study that used an animal model of endotoxemia, plasma S-nitrosothiol and hepatic nitrotyrosine levels were significantly higher in rats with cirrhosis than in control rats, and there was strong positive staining for nitrotyrosine in immunohistochemistry analyses of the livers of rats with cirrhosis. Large amount of NO can be generated by inducible NO synthase (iNOS) in the liver, via immunological stimuli, such as bacterial lipopolysaccharide (LPS) and inflammatory cytokines, which implicated in many liver diseases, including liver fibrosis. In human, Koruk et al. reported that the elevated serum NO level (determination by the stable end products of NO radical, nitrite [NO2–] and nitrate [NO3–]) in patients with hepatic cirrhosis suggesting NO contributes to the progression of cirrhosis. The levels of NO derivatives, as mentioned above, nitrotyrosine, and nitrosothiols, are also increased in autoimmune hepatitis, and primary biliary cholangitis. In NASH patients, both iNOS and nitrotyrosine levels are significantly elevated. These findings suggest a major role for NO in chronic liver injury-associated fibrogenesis.

A causative role for oxidative stress in liver fibrogenesis has been strongly suggested by several reports that supplementation with antioxidants prevents fibrogenic progression. Experimental models of liver fibrosis/cirrhosis have been used to evaluate antioxidant compounds such as polyunsaturated phosphatidylcholine, peroxisome proliferator-activated receptor (PPAR) α ligand, ursodeoxycholic acid, and resveratrol. Clinically, the only drug used to treat acetaminophen overdose patients is the precursor of glutathione (GSH), N-acetylcysteine. Other antioxidant treatments include GSH, resveratrol, or Mito-TEMPO, a Mn superoxide dismutase mimetic. S-adenosylmethionine, silymarin, and vitamin E have been tested in liver fibrosis/cirrhosis patients. An effect of vitamin E has been reported in ASH- or NASH-induced fibrosis, where histological findings such as steatosis, inflammation, and fibrosis were improved. The most promising results were reported from PIVENS trial, which was performed in 247 patients for 96 months. Vitamin E treatment led to clear histological regression without fibrosis progression. Currently, the ClinicalTrials.gov website lists 14 early and phase I–IV clinical trials of antioxidant therapies for liver cirrhosis. In these studies, vitamins, particularly vitamin E, are the most frequently studied antioxidants used as dietary supplements.

**CYGB scavenges ROS and RNS**

**Cygb holds intrinsic O2-binding capacity**

As a member of the globin family, the heme iron of Cygb has the same affinities for exogenous ligands and the same equilibrium constant for oxygen as Mb. Cygb contributes to intracellular O2 supply, acting as an O2 reservoir or as a signal transducer in O2-sensing pathways. A change in the cellular redox state could promote conformational changes in Cygb and increase O2 release (e.g., the reduction of S-S bridges by reducing agents H+ or nicotinamide adenine dinucleotide). The most promising results were reported from PIVENS trial, which was performed in 247 patients for 96 months. Vitamin E treatment led to clear histological regression without fibrosis progression. Currently, the ClinicalTrials.gov website lists 14 early and phase I–IV clinical trials of antioxidant therapies for liver cirrhosis. In these studies, vitamins, particularly vitamin E, are the most frequently studied antioxidants used as dietary supplements.

**Cygb scavenges ROS**

Cygb has several roles, including detoxification of ROS, involvement in NO metabolism, protection from apoptosis, and lipid metabolism. Plasma-produced ROS/RNS can oxidize Cygb proteins, leading to a conformational change, thereby enabling access to the heme and facilitating ligand binding. Interestingly, human Cygb mutants with one or both terminal domains truncated show slightly higher superoxide scavenging activity than wild-type Cygb. Overexpression of Cygb under conditions of oxidative stress have been found in a number of studies. Its overexpression protected human neuroblastoma SH-SY5Y cells from H2O2-in-
duced cell death\textsuperscript{100,101} and rescues the human neuronal cell line TE671 from pro-oxidant Ro19-8022-induced DNA damage.\textsuperscript{102} Furthermore, \textit{in vitro} and \textit{in vivo} overexpression of Cygb in rat HSCs protect these cells against oxidative stress and inhibit their differentiation into an active phenotype.\textsuperscript{103} Therefore, the question is whether oxidative stress is magnified in the absence of Cygb. We generated Cygb-knockout mice and challenged them with various factors that induce liver diseases. First, the mice were treated with 0.05 ppm diethylnitrosamine, an established liver carcinogen, for 36 weeks. Liver tumors occurred in 57.1\% of the knockout mice compared to 0\% of the wild-type mice. In this model, background liver tissues of knockout mice showed marked development of liver fibrosis, augmented inflammatory reactions, and overproduction of ONOO\textsuperscript{\textsuperscript{104}}. Second, mice were given a choline-deficient L- amino acid-defined diet for 32 weeks to induce steatohepatitis. Unexpectedly, 100\% of Cygb-knockout mice developed multiple liver tumors, compared to 0\% of the wild-type mice. Again, background liver tissues showed development of liver fibrosis and augmented inflammatory reactions, accompanied by DNA double-strand breaks (γH2AX expression) in hepatocytes. These results suggest a protective role for Cygb against oxidative stress, liver fibrosis, and cancer development in the presence of chronic inflammation.\textsuperscript{102} Recently, Latina et al.\textsuperscript{105} reported that the Cygb gene is transcriptionally regulated by ΔNp63 in primary epithelial cells (keratinocytes) and in cancer cells (H226, MCF-7) under both normal proliferation conditions (normoxia) and following oxidative stress. Taken together, these reports suggest that, in addition to its function as a gas carrier, Cygb acts as a cytoprotective molecule under hypoxia and oxidative stress.

**Nitric oxide scavenger**

Many globins, including Cygb, show NO dioxygenase activity.\textsuperscript{106-108} In the oxy-ferrous state, all human Ngb and Cygb, rice nsHb (riceHb1), \textit{Synechocystis} Hb (cyanoglobin, SynHb), and horse heart Mb rapidly destroy NO \textit{in vitro}, and Cygb has the highest consumption rate.\textsuperscript{109} At a low O\textsubscript{2} level (0–50 mM), Cygb with cellular reductants regulates the NO consumption rate in response to changes in O\textsubscript{2} concentration and is around 500-fold more sensitive to changes in the O\textsubscript{2} level than Mb.\textsuperscript{110} Indeed, the NO dioxygenase activity of Cygb is rapid with or without a disulfide bond; however, binding of the distal histidine following dissociation of the nitrate is affected by the presence or absence of the disulfide bond.\textsuperscript{111} The NO scavenging function of Cygb protects the NO-sensitive aconitase, and decreases ONOO\textsuperscript{\textsuperscript{−}} formation.\textsuperscript{108} Cygb plays a critical role in the regulation of vascular tone and blood pressure via NO metabolism.\textsuperscript{112} Importantly, CYGB is expressed in vessels primarily in differentiated medial vascular smooth muscle cells, where it regulates neointima formation and inhibits apoptosis after injury.\textsuperscript{113} Moreover, when the Cygb-KO mice were challenged with bile duct ligation (BDL) induced liver cholestasis, liver

![Figure 4. Loss of Cygb promoted bile duct ligation induced liver cholestasis. Bile duct ligation was performed in WT (BDL-WT) and Cygb-KO (BDL-KO) mice. Liver tissues from 1 week of BDL stained with H&E, and Sirius-Red and Fast Green (SiR-FG) showed marked hepatocyte damage and severe fibrosis in KO compared with WT mice. Immunofluorescent staining of Cygb (red) showing the absence of Cygb in KO liver. Both iNOS and HO-1 (red), the markers of RNS and ROS, respectively, revealed strong oxidative stress took place in the KO mice after 24 hours of BDL. DAPI, blue, was used as nuclear counterstain. Original magnification, \texttimes400. H&E, Hematoxylin and Eosin; Cygb, cytoglobin; iNOS, inducible nitric oxide synthase; RNS, reactive nitrogen species; ROS, reactive oxygen species; DAPI, 4',6-diamidino-2-phenylindole.](image-url)
injuries including hepatocyte damage, oxidative stress, and fibrosis were massively developed compared to corresponding WT (Fig. 4). This severe liver cholestasis is accompanied by markedly increased apoptosis cell dead as indicated in Figure 5. Furthermore, the levels of nitrite and nitrate in the serum, urine, and liver in Cygb-deficient mice are all significantly elevated. Interestingly, treatment of NO inhibitor to BDL-treated Cygb-KO mice can ameliorate this cholestasis condition. Thus, the NO-scavenging function of Cygb is crucial for protecting cells/tissues from NO accumulation.

CYTGB SUPPRESSES HSC ACTIVATION AND FIBROSIS DEVELOPMENT

HSCs and hepatic fibrosis

HSCs reside in the space of Disse, between the basolateral surface of hepatocytes and the anti-luminal side of sinusoidal endothelial cells, and contain retinoid and lipid droplets. Under physiological conditions, HSCs exhibit a quiescent phenotype and express neural markers, such as glial fibrillary acidic protein, synemin, synaptophysin, nerve growth factor receptor p75, desmin, CD146, and hepatocyte growth factor. The notion that HSCs are a major collagen-producing cell type in the normal liver was experimentally verified in 1984 by Senoo et al. and in 1985 by Friedman et al. Friedman also pointed out that normal HSCs exhibit not only fibroblastic characteristics but also smooth muscle cell-like features, such as the production of basement membrane collagen (type IV collagen) and the expression of the intermediate filament protein desmin. Beyond their role as the major collagen-producing cells, their activation is a key issue in liver fibrosis. Stimuli such as oxidative stress signals (reactive oxygen intermediates), apoptotic bodies, LPS, and paracrine signals from neighboring cells including KCs, liver sinusoidal endothelial cells, and hepatocytes can trigger HSC activation or transdifferentiation into myofibroblast-like cells that acquire contractile, proinflammatory, and fibrogenic properties. The dead or dying hepatocytes as well as leukocytes phagocytosing the cells release inflammatory mediators, damage-associated molecular patterns or danger signals, which initiate and perpetuate a non-infectious "sterile" inflammatory response. Among such mediators, tumor necrosis factor (TNF), interleukin (IL) 6, IL-1β, ROS, hedgehog ligands, and nucleotides contribute to the initiation of HSC activation. Other key elements critical for the fibrotic activity in HSCs are NADPH oxidase (NOX) enzymes, in which all NOX1, NOX2, and NOX4 are upregulated in activated HSCs compared to quiescent HSCs. Indeed, the mRNA for the cytoplasmic factor p47phox and the cell membrane proteins NOX2 and NOX1 are
detected at very low levels in quiescent HSCs by real-time Reverse Transcription Polymerase Chain Reaction, while they are highly expressed following HSC activation in culture and in cells freshly isolated from patients with liver fibrosis.\textsuperscript{190}

The pathways most involved in HSC activation and deactivation are related to membrane receptor signaling, including transforming growth factor beta (TGF\(\beta\)), which has an autocrine positive feedback loop that drives fibrogenesis via mothers against DPP homolog (SMAD) 2/3;\textsuperscript{131,132} platelet-derived growth factor, a potent chemoattractant induced during initiation of HSC activation that enhances inflammatory and fibrogenic responses;\textsuperscript{133,134} and connective tissue growth factor and epidermal growth factor receptors, which are overexpressed and phosphorylated in activated HSCs.\textsuperscript{135-138} A recent study\textsuperscript{139} identified transcription factors that prevent activation of HSCs and promote fibrosis resolution, including E26 transcription-specific transcription factor (EST1), ETS2, GATA binding protein (GATA4), GATA6, interferon response factor (IRF) 1, and IRF2. In particular, GATA6 and PPAR\(\gamma\) are required for inactivation of human HSCs and regression of liver fibrosis in mice.\textsuperscript{139} Further analyses of the metabolic changes in HSCs during the initial and chronic phases of fibrosis have provided insight into the metabolic regulation of HSC activation including metabolism of retinol,\textsuperscript{140,141} lipid,\textsuperscript{142,143} nitrogen,\textsuperscript{144} redox biology,\textsuperscript{128,145} and endoplasmic reticulum stress,\textsuperscript{146} which are important for development of targeted interventions to reverse HSC activation or trigger their apoptosis.\textsuperscript{147} Clinical and experimental studies have demonstrated that the regression of liver fibrosis may be caused by the disappearance of activated HSCs/myofibroblasts by apoptosis,\textsuperscript{126,148} inactivation into a quiescent-like state,\textsuperscript{149,150} or senescence.\textsuperscript{151,152}

**Cygb regulates HSC activation and fibrogenesis**

The ROS-scavenging function of Cygb is evidenced by its ability to detoxify radicals via reaction with its heme.\textsuperscript{153} In Xu et al.,\textsuperscript{103} overexpression of Cygb protected primary rat HSCs against oxidative stress, as assessed by reduced production of malondialdehyde and 4-hydroxynonenal, biomarkers of lipid peroxidation. In Stone et al.,\textsuperscript{154} Cygb expression was correlated with a more quiescent phenotype of stellate cells in culture and Cygb was regulated by the extracellular matrix through integrin signaling in a manner dependent on activation of focal adhesion kinase. Consistently, in human liver tissues damaged by HCV infection at various fibrosis stages, the number of Cygb-positive cells decreases with fibrosis progression.\textsuperscript{37} Interestingly, Cygb is abundant in HSCs but absent in myofibroblasts rich in fibrotic septum and positive for \(\alpha\)-smooth muscle actin (\(\alpha\)SMA), fibulin-2 and Thy-1.\textsuperscript{37} In other fibrosis conditions, overexpression of Cygb in human tendon fibroblasts decreases the expression levels of fibronectin, collagen I, collagen III, TGF-\(\beta\)1 and hypoxia-inducible factor 1, suggesting an anti-scarring effect of Cygb post-glaucoma surgery.\textsuperscript{155} Randi et al.\textsuperscript{156} demonstrated abundant Cygb expression in human podocyte lines and that it has an antioxidant effect in chronic renal diseases.

We have generated, for the first time, a new transgenic (TG) mouse line in which both Cygb and mCherry reporter gene expression is controlled under the native Cygb gene promoter. Administration of a single dose (50 mg/kg) of thioacetamide (TAA) in Cygb-TG mice resulted in lower levels of serum alanine aminotransferase and oxidative stress than those of wild-type mice. At 10 weeks of TAA administration, Cygb-TG livers exhibited reduced neutrophil accumulation, cytokine expression and fibro-
sis, and high levels of quiescent HSCs.\textsuperscript{157} HSCs in the absence of Cygb (HSCs\textsuperscript{Cygb-null}) become enlarged with a developed αSMA network after 7 days in culture and lose cellular lipid droplets more rapidly than HSCs\textsuperscript{Cygb-wild}. Moreover, HSCs\textsuperscript{Cygb-null} shows a pre-activated phenotype with increased oxidative stress and markedly elevated expression of cytokines and chemokines such as IL-6, TNFα, IL-1β, chemokine (C-X-C motif) ligand-1, -2, and chemokine ligand-2, -3, -4.\textsuperscript{52} By contrast, primary HSCs isolated from Cygb-TG mice (HSC\textsuperscript{Cygb-TG}) exhibit a significantly decreased αSMA, collagen 1α1, and TGF-β3 after 4 days in culture compared to WT cells. HSCs\textsuperscript{Cygb-TG} are resistant to H\textsubscript{2}O\textsubscript{2}-induced αSMA expression.\textsuperscript{157} Thus, cell-specific overexpression of Cygb attenuates HSC activation and protects mice against TAA-induced liver fibrosis presumably by maintaining quiescence of HSCs. Figure 6 shows the role of Cygb in liver fibrosis.

**CONCLUSION AND FUTURE DIRECTION**

More than 600 clinical trials of antifibrotic drugs are underway (http://www.ClinicalTrials.gov). One reason for this is the recognition of the central role of HSCs in liver fibrosis. Promising approaches to removing fibrogenic cells are being evaluated, including drug-delivery systems targeting activated HSCs. In parallel, antioxidant therapy has been targeted by the recovery of antioxidant enzymes/compounds and by reducing the production of ROS and RNS. Furthermore, a number of recent studies have explored the protective role of Cygb in hepatic fibrosis, which is mediated by inactivation of HSCs. Hopefully, the best drug for anti-fibrotic therapy will be discovered in the near future.

**Authors’ contributions**

LTTT, HH, and NK contributed to the literature review, and manuscript preparation.

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**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

15. Meagher EA, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J,
27. Win S, Than TA, Kaplowitz N. Hepatic mitochondrial Sab (SH3BP5) plays a pivotal role in sustained JNK activation and steatohepatitis in diet-induced NASH. Hepatology 2016;64:128A.
Cytoglobin suppresses HSC activation and fibrosis

Le Thi Thanh Thuy, et al.


122. Reeves HL, Friedman SL. Activation of hepatic stellate cells--a key issue in liver fibrosis. Front Biosci 2002;7:d808-d826.

Fecal microbiota transplantation in alcohol related liver diseases

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The current standard of care for severe alcoholic hepatitis (SAH) has several limitations in that only up to one-third of patients are eligible for steroid therapy. Additionally, steroids have their own issues: a portion of patients do not respond, while there is doubtful long-term benefit in those who do and a large proportion are ineligible to receive steroids entirely and hence have no definitive options for treatment. As such, there is a large gap between the problem and the available solutions. Alcohol causes dysbiosis and also disrupts gut barrier function, consequently promoting the translocation of microbial lipopolysaccharide into the portal circulation and liver. Therefore, probiotics, prebiotics, antibiotics, or transplantation of gut microbiota are likely to attenuate the dysbiosis-related liver insult. Fecal microbiota transplantation (FMT) is expected to have a role in managing alcoholic liver disease in general and SAH in particular by correcting dysbiosis, the primary insult. Results from mouse studies have suggested beyond doubt that alcohol-related liver injury is transferrable and also treatable by adopting FMT from suitable donors. Initial human trials from our center have affirmed benefits in human subjects with SAH as well, with both improvements in disease severity and as well as the rate of survival. Further studies addressing the head-to-head comparison of steroids and FMT are ongoing. Available preliminary data are promising and FMT and/or gut microbial modulation might become the standard of care in the near future for managing alcohol-related liver diseases, especially alcoholic hepatitis, with greater applicability, improved acceptability, and minimal side effects. (Clin Mol Hepatol 2020;26:294-301)

Keywords: Fecal microbiota transplantation; Alcoholic liver diseases; Alcoholic hepatitis

INTRODUCTION

In recent years, we have seen increasing evidence regarding the role of gut microbiota in the pathogenesis and progression of many diseases. The liver, being the first line of filtration between the gut and the rest of the body, is exposed to the brunt of changes in the gut microbiome and hence is more likely to be affected by dysbiosis. It is likely that most liver diseases are responses of the liver to such changes in the gut microbiome. The gut microbiome can be affected by the food we eat, drugs we take, and other aspects of our lifestyle including alcohol intake.

In correlation with changes in the lifestyle and improvements in the financial status, alcoholic liver disease has become the most common chronic liver disease, even in Asian countries. Nearly 1% of the global deaths and 50% of the cases of liver disease worldwide are caused by alcohol. Alcohol hepatitis, the most florid form of alcoholic liver disease, has a very high short-term mortality of up to 50% and no specific therapies are available other than...
steroids. Steroids also only show a limited utility in improving the short-term survival and boast no evidence of any long-term benefits. Additionally, only a small proportion of patients with alcoholic hepatitis are eligible to receive steroids. Thus, a large number of patients are either not eligible or do not respond to steroids and this group outnumbers those who do respond to steroids, leaving us without any specific therapeutic options for a majority of these individuals. Even liver transplantation is not feasible in most cases due to the presence of sepsis or recent alcohol consumption and many ethical and logistic issues are involved despite the documented safety and survival benefits of early liver transplantation in patients with severe alcoholic hepatitis (SAH) not responding to medical management. Therefore, newer, more effective, and nontransplant therapeutic options for managing severe alcoholic hepatitis are needed.

Since gut dysbiosis, leaky gut, and products of the gut microbiome reaching the liver are the main culprits in the development of alcoholic hepatitis, targeting qualitative and quantitative changes in the gut microbiome remains an important strategy in developing new therapies for alcoholic hepatitis. Among others, the modulation of gut microbiota by fecal microbiota transplantation (FMT) has recently been conceptualized and evaluated as a potential therapeutic strategy in both preclinical and clinical studies.

The methods for evaluating the gut microbiota have also remarkably improved in recent years. Next-generation sequencing of bacterial DNA has helped in assessing the bacterial composition and community diversity from the phyla to the species level, without the need for cultures. Sequencing of the bacterial ribosomal RNA gene (16s rRNA) is widely available for assessing specific bacterial taxa and their relative abundances by referencing from online databases. Metagenomic analysis by the shotgun approach can assess all the genes present in a given sample and identify abundances of specific metabolic processes. In this review, we discuss the current status of FMT in managing alcoholic hepatitis and alcoholic liver disease.

**THE GUT MICROBIOME AND ITS ROLE IN THE PATHOGENESIS OF ALCOHOLIC LIVER DISEASE**

Prolonged alcohol consumption results in the development of fatty liver (due to increased fatty acid/triglyceride synthesis and as well as increased fatty acid influx), while additional unknown triggering events initiate steatohepatitis. Gut dysbiosis, greater gut permeability, and increased gut microbial products in the portal circulation are the most commonly reported triggers initiating al-

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Figure 1. Role of the gut-liver axis in the pathogenesis of alcoholic hepatitis/alcoholic liver disease and potential mechanisms of action of FMT in the management of alcohol-associated liver diseases. LPS, lipopolysaccharide; IL-6, interleukin-6; IL-8, interleukin-8; TNF-α, tumor necrosis factor-alpha; ROS, reactive oxygen species; FMT, fecal microbiota transplantation.
cohoic hepatitis, prompting the activation of the innate and adaptive immune systems, hence the hepatic insult. Some questions like why only a small proportion of alcohol consumers develop liver disease and an even lesser proportion develop alcoholic hepatitis might have answers in the gut microbiome signatures they possess. Gut microbiome modulation may be an important and promising way of dealing with alcoholic liver disease (Fig. 1).

Normally, the gut microbiome boasts significant diversity, including bacteria, fungi, and viruses. Bacteria itself have multiple phylae, with many families, genera, and species. Given the presence of such a diverse population of organisms in the gut, with more than 150 times the active genes in the human body itself, and, together with its varied metabolites, the gut cannot just be a silent organ but rather holds an integral role in health and various diseases. Whenever the symbiotic relationship between the host and the gut microbiome is unbalanced (dysbiosis), the products from the gut contribute to the development of disease states, with alcoholic liver disease being one example.

Alcohol consumption causes disruption of the symbiosis between the gut microbiota and the host (dysbiosis), with additional disruption of the intestinal barrier, leading to the onset of leaky gut, as assessed by increased serum endotoxin levels in patients with alcoholic liver disease and alcoholic hepatitis. The causal role of gut microbial changes in the development of alcoholic liver disease has recently been assessed in many preclinical and clinical trials. Translocation of viable bacteria, bacterial metabolites, translocation of pattern-associated molecular patterns, intestinal inflammation, and changes in bile acids have been proposed as mechanisms of the onset and progression of alcohol-associated liver disease. Prolonged alcohol consumption is known to decrease the beneficial lactobacillus species irrespective of the presence of cirrhosis. Increases in proteobacteria and Fusobacteria and reductions in Lactobacillus and Bacteroidetes species are also well-established in alcoholic cirrhotics. Ethanol consumption has also been shown to be correlated with increases in the abundance of endotoxin-producing Enterobacteriaceae and a reduction in taxa that produce short-chain fatty acids such as Lachnospiraceae and Ruminococcaceae. An increase in the abundance of Candida species and decreased fungal diversity have also been observed in patients with alcoholic hepatitis. Alcohol consumption is shown to increase the bacterial colony counts (both aerobic as well as anaerobic), including more significantly in the proximal small bowel. In recent studies, it has been demonstrated that FMT from alcoholic hepatitis patients can produce necro-inflammatory changes in germ-free mice, proving the pathogenetic role of alcohol-related gut dysbiosis in the development of liver injury.

In addition to the occurrence of dysbiosis, changes in the fecal metabolites, such as reductions in short-and long-chain fatty acid levels and increases in the levels of both total and conjugated bile acids, are known to modulate the gut-liver axis in alcoholic liver disease. Disruption of the mucosal barrier function prompting changes in the mucus layer, reductions in secreted antimicrobial proteins like REG3G and mucin-2, the disruption of tight junctions leading to leaky gut, and alterations of the innate and adaptive immunity in the subepithelial space of the intestines have also been strongly linked to the pathogenesis of alcohol-induced liver disease.

CURRENT STATUS OF GUT MICROBIOME MODULATION IN ALCOHOL-ASSOCIATED LIVER DISEASES

The gut microbiome is a very pliable environment that can be modulated with diet; pre-, pro- or antibiotics; and FMT. In preclinical trials, probiotics (Lactobacillus, Bifidobacterium) have been shown to improve alcohol-induced liver inflammation and gut leakiness. Prebiotics like fructo-oligosaccharides, which are substances that increase the number of beneficial gut microbes, improved alcohol-induced liver damage in mice. Interestingly, fecal microbiota manipulation by pectin, a fiber present in fruits, restored Bacteroides levels in mice and thus prevented liver injury by alcohol.

Research suggests intestinal and circulating mucosa-associated invariant T (MAIT) cells are altered and involved in the increased number of bacterial infections present in patients with SAH. As such, FMT is likely to alter the MAIT cell dysfunction in SAH. Alcohol-sensitive mice were found to have decreased Bacteroidetes and increased Firmicutes and Actinobacteria populations relative to alcohol-resistant mice procured from the same laboratory. FMT from the alcohol-resistant mice to the alcohol-sensitive mice reversed the Bacteroidetes depletion and protected the mice from alcohol injury. In another translational study, Llopis et al. used germ-free humanized mice and completed FMT from alcoholic liver disease patients with and without SAH. With consequent alcohol feeding, the mice with FMT from SAH patients showed more severe liver inflammation, greater hepatic necrosis, higher intestinal permeability, and translocation of bacteria when compared with the mice which received FMT from non-SAH alcoholic patients. Also, the microbiome composition was distinctly different
between the SAH and non-SAH alcoholic hepatitis patients as revealed by principal component analysis. Subsequently, a second FMT from patients without SAH into the mice who had earlier received FMT from SAH patients yielded improvements in liver lesions, confirming the etiopathological and therapeutic roles of the gut microbiome in severe alcoholic hepatitis. Current human data on FMT for alcohol-associated liver diseases are sparse; the available information along with the FMT preparation process will be discussed in detail in the subsequent sections.

**FMT IN ALCOHOLIC LIVER DISEASE**

Coprophagy (consumption of feces) is common in the animal kingdom. Transfaunation has been practiced for centuries in veterinary medicine for treating conditions like ruminal acidosis and chronic diarrhea. Transplanted fecal material from donors may possibly preserve thousands of functional bacterial species and eventually re-establish a healthy functional gut microbiome in the recipient. The concept of FMT is not new: traditional Chinese doctors in the fourth century AD described the use of FMT (orally) for treating patients with difficult diarrheas. The last decade has seen a resurgence in the utility of FMT in managing human diseases with the United States Food and Drug Administration approving FMT for managing difficult-to-treat *Clostridium difficile* infections.

**FMT process**

**FMT donation and processing**

Donor screening is of the utmost importance and should identify a healthy donor without any active infections by conducting screenings for hepatitis B surface antigens, anti-hepatitis C antibodies, human immunodeficiency viruses 1 and 2, and venereal disease research laboratory testing. The donor’s stool should also test negative for ovarian cysts, Rotavirus antigens, *Helicobacter pylori* antigens, *Cryptosporidium*, *Isospora* (AFB stain), and *C. difficile* toxins. Donors should have not experienced recent alcohol intake (in the last 90 days), altered bowel movements, or recent antibiotic use (in the last 90 days) (Table 1). The donor should also be free of any significant co-morbidities or chronic ailments and preferably aged between 18 and 60 years. In general, a young, healthy, lean, individual, preferably a relative (who possibly would share the same kitchen/human leukocyte antigen alleles), is an ideal donor for FMT. A stool sample should be collected in the early morning a clean plastic container, preferably at a place very near to the processing unit (the FMT laboratory) to be processed in an automated system for safe and early disposal of the filtrate containing the microbiome suspension without fibrous residue.

**Fecal preparation for FMT**

Fresh fecal samples are preferred over frozen or stored samples given safety, efficacy, and viability concerns. Some reports suggest the level of efficacy is equal between the stored samples and

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<td>Stool modified ZN stain (<em>Cryptospora</em> and <em>Isospora</em>)</td>
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<tr>
<td><em>Clostridium difficile</em> antigens and toxins</td>
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<td>Rotavirus antigens</td>
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<td>HBsAg, anti-HCV, HIV 1 and 2</td>
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<td>VDRL</td>
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Table 1. Donor screening for FMT

Donors were excluded if they had any one of the following conditions or characteristics:

- Abnormal bowel motions
- Obesity
- Chronic alcohol intake
- Active substance abuse or failed to provide consent
- Age of less than 18 or more than 60 years
- HBsAg, anti-HCV, HIV seropositivity
- Gastroenteritis within the last 2 months
- Inflammatory bowel disease
- Current or past history of any malignancy
- Diabetes, chronic kidney disease, coronary artery disease, stroke, COPD
- Antibiotic usage within 3 months at the time of enrolment
- Elevated liver enzyme

FMT, fecal microbiota transplantation; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C virus; HIV, human immunodeficiency virus; COPD, chronic obstructive pulmonary disease; VDRL, venereal disease research laboratory testing.
the stored frozen samples but most prefer fresh samples due to concerns about the loss of a proportion of the viable microbiome. At many large-volume FMT centers, including ours, the FMT workflow (collection, transportation, preparation, and delivery) is designed to be completed within 3 hours and fresh stool samples are always preferred, although further research in this regard is warranted. Automated GenFMTer purification systems (FMT Medical, Nanjing, China) are also useful in facilitating clean handing of the samples and packaging of the fecal slurry in easily and safely transportable parcels.

Routes of administration

FMT route of administration

As of today, the best route of administration is not confirmed from amongst the oral, nasogastric, nasoduodenal, nasojejunal, endoscopic, rectal, and colonoscopic options depending on the disease condition and there are no head-to-head comparisons available in the literature. However, in cirrhotics with dysbiosis predominantly involving the proximal small bowel, the upper gastrointestinal route of FMT administration seems more logical. At our center, we prefer adopting nasoduodenal tube placement for FMT instillation in our cirrhotics to avoid the risk of aspiration.

Current data for FMT in ALD

We now know that the alcohol induces dysbiosis, including more so in the proximal small bowel, and preclinical studies have confirmed the role of gut microbiota modulation to prevent or improve liver injury by alcohol. However, the precise method of gut modulation and the optimal site, duration, and method of modulation are still less clear, with many human trials still in the preliminary stages. With the immense potential of modulation of the gut microbiota and its possible therapeutic implications, researchers to date have attempted FMT in patients with severe alcoholic hepatitis, hepatic encephalopathy, hepatitis B-related chronic liver diseases, and nonalcoholic fatty liver disease.

In one clinical pilot trial involving patients with SAH (n=8), Phil...
ips et al.\textsuperscript{35} administered FMT consecutively for seven days in steroid-ineligible patients and reported improvements at one year regarding survival in comparison with historical controls (87.5\% vs. 33.3\%). Both the relative abundance of \textit{Proteobacteria} and a low abundance of \textit{Actinobacteria} at baseline in SAH patients improved after FMT. Additionally, the coexistence of recipient and donor species was noted even at 6 to 12 months after FMT. This coexistence reiterates the fact that the donor bacteria modulate the pre-existing recipient bacteria and suppresses their pathological nature by possibly increasing the biodiversity. In fact, changes have also been observed at the species level with a reduced abundance in the pathogenic species (\textit{Klebsiella pneumoniae}, from 10\% to 1\% by 1 year) and an increased relative abundance of beneficial species (\textit{Enterococcus villorum}, \textit{Bifidobacterium longum}, and \textit{Megasphaera elsdenii}). At the metagenomic level, bile secretion, carotenoid synthesis, and pantothenate synthesis pathways, which were downregulated at baseline, improved during follow-up after FMT. This trial highlighted the safety of FMT even in the sickest group of SAH patients (i.e., steroid-ineligible patients) and further paved the way for future trials. Following this trial, a randomized controlled trial (NCT 03091010) involving the comparison patients with SAH receiving steroids or FMT from our center showed promising results, with an improved 90-day survival rate in the group receiving FMT relative to those given steroids revealed by the preliminary unpublished data (n=112). Further detailed results of this trial will be of the utmost importance and may change the way we currently treat our patients with SAH (Table 2).\textsuperscript{35,37-40} To our knowledge, in the currently available literature, no major side effects attributed FMT have been described. Only abdominal distension due to gaseous bloating has been documented in a small proportion of patients.

Dysbiosis and the gut-liver axis are important and integral parts of the pathogenesis, progression, and outcomes of most liver diseases and even more so with respect to alcohol-associated liver diseases. Despite the immense potential perceived, current approaches in the modulation of the gut microbiome as therapies for liver disorders are inadequate. We must improve our protocols for FMT preparation, transport, and delivery so as to enhance both the quality (microbial diversity/richness) and shelf life of samples and alleviate aesthetic concerns to support greater con-

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**Figure 2.** Current position for FMT in severe alcoholic hepatitis. DF, discriminant function; MELD, Model for End-stage Liver Disease.
venience and improved efficacy. Also, the best route of administration according to the disease condition needs to be further explored—for example, colonic administration for difficult-to-treat *C. difficile* infections and proximal delivery for liver diseases including alcoholic liver disease where dysbiosis is more pronounced in the proximal bowel may be optimal techniques. The timing, quantity, frequency, and assessment of response to treatment all warrant continued exploration in future trials.

**CONCLUSION**

Current treatment options in the management of SAH remain primitive and rudimentary. Steroids are still the standard of care with doubtful long term benefits and only about one-third of patients with SAH are even eligible for steroids, of which only a proportion respond positively to therapy. A large number of patients with SAH are therefore either ineligible or nonresponsive. There is no blanket standard of care available to date as a standard of care to treat each and every patient with SAH that displays good long-term efficacy. In this regard, FMT stands a fair chance of attaining a position in the current SAH treatment algorithm, at least in steroid-ineligible or nonresponsive patients and in those with persistent liver failure (Model for End-stage Liver Disease score >14 points) despite the completion of steroids (Fig. 2). Further high-quality trials are required to address many unsolved issues.

**Conflicts of Interest**

The author has no conflicts to disclose.

**REFERENCES**


Liver biopsy has been referred to as the gold standard for the diagnosis of liver diseases and evaluation of liver fibrosis or liver damage. The American Association for the Study of Liver Disease guidelines have suggested the following indications for liver biopsy, namely, diagnosis of parenchymal liver diseases, abnormal liver function tests of uncertain causes, fever of unknown origin, and abnormal findings on imaging studies; staging of parenchymal liver diseases; and development of treatment plans based on histopathologic findings. Among all indications for liver biopsy, its use in diagnosis is of utmost importance. For example, histological findings of interface hepatitis and lymphoplasmacytic infiltrates in portal tract are seen in autoimmune hepatitis, and destruction of the interlobular bile duct and nonsuppurative destructive cholangitis are observed in primary biliary cholangitis. Liver biopsy plays an important role in the diagnosis of malignancies, such as hepatocellular carcinoma, which can also be diagnosed by typical imaging findings in multiphasic computed tomography (CT) or magnetic resonance imaging (MRI) in patients with risk factors, such as cirrhosis or chronic viral hepatitis. However, liver biopsy should be considered in patients who show typical image findings but do not have risk factors as well as in those who have risk factors but do not show typical image findings. Limitations to liver biopsy include invasive nature with possibility of complications, sampling variability and the subjective nature of the pathologist interpretation. Therefore, non-invasive methods have recently replaced or supplemented a significant portion of liver biopsy. As non-invasive tests, the scoring systems using serologic test such as fibrosis-4 (FIB-4) index, non-alcoholic fatty liver disease (NAFLD) fibrosis score, or aspartate transaminase to platelet ratio index (APRI) might be useful to screen advanced fibrosis or cirrhosis. These methods are easily obtained at no additional cost. Patients suspected of cirrhosis in these scoring systems might be confirmed without liver biopsy by imaging based non-invasive methods such as ultrasonography based elastography such as transient elastography and two-dimensional shear wave elastography, and magnetic resonance elastography. In the current study, the changes in indications for liver biopsy...
in a single Korean tertiary care center were evaluated. In general, conducting liver biopsies for viral hepatitis has been found to decrease over time. This finding suggested the development of non-invasive methods replacing the reported indications of liver biopsy for fibrosis staging in viral hepatitis. Transient elastography is the most widely used non-invasive method in the tertiary care center, and has been validated for the evaluation of liver fibrosis in various liver diseases.\(^7\) Filtering out chronic hepatitis B patients with advanced fibrosis or cirrhosis is very important to determine the antiviral therapy or evaluate prognosis because current guidelines generally recommend antiviral therapy in patients with cirrhosis regardless of alanine transaminase levels,\(^8,9\) and thus, non-invasive test methods can be very beneficial in determining antiviral therapy without a liver biopsy. In addition, the results of transient elastography on the degree of fibrosis can predict hepatocellular carcinoma risk in patients with chronic hepatitis B.\(^10,11\) However, the degree of liver stiffness from transient elastography can be overestimated in case of severe inflammation of liver,\(^12\) and the measurement of fibrosis may be difficult in patients with obesity or ascites. Therefore, it should be interpreted in consideration of various situations. Similar to viral hepatitis, liver biopsies for malignancy were also decreased over time in the current study,\(^6\) and it seems to be possible to diagnose hepatocellular carcinoma in most patients who have risk factors such as viral hepatitis or cirrhosis without liver biopsy due to the development of CT or MRI techniques.

However, in the current study, liver biopsies for autoimmune hepatitis or primary biliary cholangitis were increased.\(^6\) Although various serologic markers may suspect autoimmune hepatitis, but most cases are confirmed by liver biopsy.\(^7\) For primary biliary cholangitis, liver biopsy is especially helpful when anti-mitochondrial antibody is negative, or overlap syndrome with autoimmune hepatitis is suspected. In primary sclerosing cholangitis, “onion skin” can be observed in the biopsy sample. These pathological findings cannot be replaced by non-invasive tests. Non-invasive tests are helpful in NAFLD—transient elastography can measure not only fibrosis but also steatosis by controlled attenuated parameter. Despite the availability of non-invasive methods in the current study, liver biopsies for NAFLD also showed an increased pattern.\(^6\) This could have been due to an increased incidence of NAFLD, including non-alcoholic steatohepatitis (NASH) in recent years. A liver biopsy is considered as an important test if the degree of steatohepatitis or NAFLD activity score (NAS) needs to be determined because there has been no proven non-invasive test that reflects the degree of inflammation and fibrosis in NASH. A liver biopsy also provides us useful informations when the cause of hepatitis is not clear. There is no alternative method or test that can replace liver biopsy for demonstrating the actual status of liver disease.

Percutaneous liver biopsy is the most widely used approach and is currently performed under ultrasonographic guidance. Conducting liver biopsy under guidance from ultrasonography has reduced the risk of complications.\(^3\) Two retrospective studies reported that major adverse events after percutaneous liver biopsies were about 1.0%.\(^14,15\) Pain was the most common post-biopsy complication that occurred in 30–50% of the patients,\(^16\) but was not usually serious. Bleeding was the most common serious complication reported to occur in 0.6% of the patients.\(^15\) Rare complications of organ injury, such as pneumothorax, hemothorax, or bile peritonitis have also been reported.\(^1\) The outcomes in the current study showed a comparatively lower incidence of major adverse events (0.05%) than previous studies.\(^6\) This study included patients with advanced cirrhosis and ascites belonging to Child-Pugh Class B or C. Study results suggested that ultrasonography-guided percutaneous liver biopsies were performed without serious complications in most patients, including advanced cirrhosis; however, this study did not record whether patients with advanced cirrhosis had any bleeding tendency, such as thrombocytopenia or prolongation of prothrombin time. Therefore, in cirrhotic patients with bleeding tendency, other approaches, such as transjugular liver biopsy should be considered.

Although various non-invasive methods have been developed and are currently available, liver biopsy has its own irreplaceable role and is still important. The indication of liver biopsy has been changed from assessment of fibrosis in viral hepatitis to diagnosis of autoimmune hepatitis, primary biliary cirrhosis, and NASH mainly due to the changes in disease incidence as well as due to the development of non-invasive test. A liver biopsy can be safely performed in most patients, and therefore, should be always considered, if necessary.

**Conflicts of Interest**

The authors have no conflicts of interests to disclose.

**REFERENCES**

2. Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL,
Optimal sequence of systemic therapy after sorafenib failure in patients with hepatocellular carcinoma

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Keywords: Carcinoma, Hepatocellular; Sorafenib; Protein kinase inhibitors

Hepatocellular carcinoma (HCC) is one of the solid cancers with a dismal prognosis, particularly when it is diagnosed in the unresectable/advanced stage. Owing to the increasing incidence of HCC in developed countries over the last decade, there has been a remarkable progress in the development of novel drugs for systemic treatment, enabling HCC to escape from being classified as an “orphan tumor.” Indeed, several tyrosine kinase inhibitors, including sorafenib, lenvatinib, regorafenib, and cabozantinib, have been approved as first- or second-line systemic therapies for patients with unresectable HCC. Furthermore, nivolumab, which is an immune checkpoint inhibitor targeting programmed cell death protein 1 (PD-1) on cytotoxic T cells, and ramucirumab, a monoclonal antibody inhibiting vascular endothelial growth factor receptor 2 (VEGFR2), have been approved as second-line systemic therapies with an action mechanism different from that of tyrosine kinase inhibitors. With various systemic therapeutic options now available, liver specialists treating patients with HCC have to answer a new question: what is the optimal second-line systemic treatment option after sorafenib failure?

Regarding this issue, Lee et al. reported comparable efficacy and safety between nivolumab and regorafenib in patients with advanced disease (Barcelona Clinic Liver Center [BCLC] stage B or C) who had experienced sorafenib failure. Their retrospective study was conducted at a single institution, and most of the enrolled patients had BCLC stage C disease (96.7%). In 102 and 48 patients who were treated with nivolumab and regorafenib, the median overall survival (OS) was 5.9 months and 6.9 months, respectively (P=0.77). There was no significant difference in disease control rates between the nivolumab and regorafenib groups (50.0% vs. 47.1%; P=0.58), but the objective response rate with nivolumab was higher than that with regorafenib (16.7% vs. 5.9%; P=0.041). There was no significant difference in time to progression (TTP) between the two groups (4.0 vs. 3.3 months; P=0.4). The median OS and TTP of regorafenib-treated patients in the study were lower compared to the RESORCE trial, possibly due to the larger proportion of patients with macrovascular invasion and BCLC stage C in the current study. Some patients (5.9%) on regorafenib had prior sorafenib intolerance, which may have lowered OS or TTP compared to the outcomes reported in the RESORCE trial.
RESORCE trial, as longer duration of sorafenib treatment is associated with better OS and TTP with subsequent regorafenib treatment.\textsuperscript{10} In contrast, the median OS of nivolumab-treated patients was consistent with that reported in a previous study.\textsuperscript{7} Independent prognostic factors for OS were nivolumab treatment (hazard ratio [HR], 0.536; 95% confidence interval [CI], 0.300–0.957; \(P=0.04\)), male sex (HR, 2.587; 95% CI, 1.140–5.872; \(P=0.02\)), Child-Pugh class B (HR, 5.195; 95% CI, 2.073–13.018; \(P=0.001\)), and intrahepatic tumor burden (HR, 2.801; 95% CI, 1.019–7.703; \(P=0.046\)). Regarding safety, patients treated with nivolumab or regorafenib had comparable toxicity leading to premature drug discontinuation, mostly from hepatic decompensation. The authors suggest that patients with Child-Pugh class B would tolerate nivolumab better than regorafenib, as there was no difference in discontinuation rates due to hepatic decompensation despite the larger number of patients with Child-Pugh class B in the nivolumab group (18.8% vs. 3.9%; \(P=0.003\)).

The authors applied inverse probability of treatment weighting (IPTW) to reduce treatment selection bias, which is unavoidable in retrospective studies. In fact, the baseline characteristics were not well-balanced between the two groups. In addition to the difference in sample size (n=48 in nivolumab, n=102 in regorafenib), a greater proportion of patients in the nivolumab group (18.8%) had poor liver function (indicated by Child-Pugh score 7–9) compared to those in the regorafenib group (3.9%). Additionally, the proportion of patients with intrahepatic tumor burden >50% tended to be higher in the nivolumab group (27.1%) than in the regorafenib group (18.6%), although the difference was not statistically significant \(P=0.40\). Even after IPTW, nivolumab treatment remained a significant independent factor associated with prolonged OS (HR, 0.340; 95% CI, 0.177–0.653; \(P=0.001\)). However, in the multivariate analysis after IPTW, nivolumab treatment was not found to be an independent factor related to prolonged TTP (HR, 0.744; 95% CI, 0.394–1.405; \(P=0.36\)). Based on the results obtained using IPTW, the authors concluded that nivolumab treatment might be associated with prolonged OS compared to regorafenib treatment in patients who progressed afterwards or were intolerant of sorafenib.

Although IPTW estimation is now commonly used to control for confounding factors in nonexperimental studies of medical interventions,\textsuperscript{11} not all of the confounders could be adjusted. In the study by Lee et al.,\textsuperscript{9} the median duration of sorafenib treatment was 2.5 months (1.4–3.1) and 3.0 months (2.3–6.2) in the nivolumab and regorafenib groups, respectively \((P<0.001)\). Considering that longer sorafenib treatment duration is associated with better response to regorafenib, and that physicians may prefer regorafenib to nivolumab as the second-line treatment in case of a favorable response to sorafenib, there is a possibility that selection bias which cannot be corrected by IPTW may persist. Liver function and tumor burden are the strongest prognostic factors.

**Figure 1.** Proposed algorithm for the selection of systemic treatment after sorafenib failure. Potential options for sequential systemic therapies are presented. Regorafenib was the second-line therapy for patients who tolerated sorafenib (≥400 mg of sorafenib for 20 days or longer during the 28-day period before PD) and progressed on sorafenib. Nivolumab or cabozantinib could be used for patients with intolerance to sorafenib. Ramucirumab was reserved for second-line therapy in patients with (AFP concentration ≥400 ng/mL. Cabozantinib was the only drug listed as a third-line treatment. PD, progressive disease; AFP, alpha-fetoprotein.
particularly in advanced HCC patients who have experienced systemic therapy. Ideally, it is desirable to conduct a randomized, controlled trial to compare the efficacy and safety between different types of systemic therapies, but it is unlikely that global pharmaceutical companies would proceed with such a trial, considering the high risk of a negative result. The alternative would be to collect nationwide large-scale data to mitigate biases such as the differences in sample size, tumor burden, liver function, and physicians’ preference between groups.

With the increasing availability of drugs for first- and second-line systemic therapy for HCC, a critical question among physicians will relate to treatment strategy. What would be the optimal sequence after failure of a first-line drug, such as sorafenib? How should new drugs and data be integrated into the evolving sequence paradigm? An important hurdle to overcome is the absence of useful biomarkers to guide physicians in selecting drugs for HCC, even though recently developed drugs have molecular or immunologic targets. Unfortunately, in a recent phase 3 clinical trial testing pembrolizumab, a monoclonal antibody targeting the PD-1 interaction with programmed death-ligand 1 (PD-L1), as second-line therapy for HCC patients (KEYNOTE-240), the median OS was 13.9 months (95% CI, 11.6–16.0) for pembrolizumab versus 10.6 months (95% CI, 8.3–13.5) for placebo (HR, 0.781; 95% CI, 0.611–0.998; P=0.238). Consequently, if similar experience is possible with nivolumab, which has been accepted as a second-line drug based on phase 1/2 data, the role of an immune checkpoint inhibitor as rescue therapy after sorafenib failure might not be so promising.

At present, a possible approach to a systemic treatment strategy can be suggested in light of the available data. Patients who were tolerant of sorafenib and had disease progression would be managed with regorafenib as second-line therapy, according to RESORCE trial. Cabozantinib, a multiple receptor tyrosine kinases inhibitor inhibiting VEGFR2, c-MET, and AXL, was approved as a second-line and third-line treatment for advanced HCC. In subgroup analysis, cabozantinib demonstrated favorable effects in patients aged ≥65 years, males, and those with extrahepatic spread. Patients who discontinued sorafenib due to toxicity would be considered for nivolumab, cabozantinib, or ramucirumab. Nivolumab was tested in an open-label, non-comparative, phase 1/2 dose study (Checkmate 040) that assessed the safety and efficacy of nivolumab in patients with HCC who failed sorafenib treatment or other systemic therapy and those who were intolerant to sorafenib. Ramucirumab, for which survival benefit compared to placebo is not meaningful in patients who failed or were intolerant to sorafenib (8.5 vs. 7.3 months), showed improved survival in patients whose alpha-fetoprotein (AFP) concentrations are 400 ng/mL or greater. Therefore, ramucirumab should be restricted to patients whose AFP concentrations are 400 ng/mL or greater in both sorafenib intolerant and tolerant patients (Fig. 1). However, CELSTIAL trial (phase 3 double-blind placebo-controlled trial randomizing 773 HCC patients to cabozantinib or placebo in the second- or third-line setting) also reported favorable response to this particular subgroup (AFP >400 ng/mL); therefore, uncertainty remains on the superiority of ramucirumab over other treatment agents as second-line therapy following sorafenib failure. When choosing the type of systemic therapy, it is important to consider the cost-effectiveness. One study reported that cabozantinib would not be cost-effective as the second-line therapy in advanced HCC. In the near future, the systemic treatment paradigm will be changed as lenvatinib becomes increasingly used as a first-line therapy, and the combination of atezolizumab with bevacizumab has shown promising results as the first-line treatment in a recent phase 3 trial. With rearrangement of first-line systemic therapies in practice, the need to select the optimal second-line treatment will again be raised. It remains unclear whether individual tumor biology would help to establish predictive biomarkers in HCC and to allocate the most effective drug to the right patient. Until biomarker-driven therapy is realized, efforts should be focused on identifying the special sub-cohorts of patients who respond to individual systemic treatments.

Conflicts of Interest

The authors have no conflicts of interests to disclose.

REFERENCES


Diagnosis of hepatocellular carcinoma: Which MRI contrast agent? Which diagnostic criteria?

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Keywords: Carcinoma, Hepatocellular; Diagnostic imaging; Magnetic resonance imaging

Of late, there have been substantial changes to the imaging-based diagnosis systems for hepatocellular carcinoma (HCC) in high risk patients; the Korea Liver Cancer Association-National Cancer Center (KLCA-NCC),1 the American Association for the Study of Liver Disease (AASLD),2 and the European Association for the Study of the Liver (EASL)3 all released updated guidelines for HCC in 2018. One of the most prominent changes in these latest updates is the active adoption of hepatobiliary contrast agent (HBA) for magnetic resonance imaging (MRI) of the liver, besides the conventional extracellular contrast agent (ECA). This change has fueled much recent research regarding diagnostic criteria and the proper selection of MRI contrast agent.

In the past, when ECA was the only choice, diagnostic criteria were simple and the same across the globe: arterial phase hyper-enhancement and washout during the portal or delayed phases. After the introduction of HBA, the diagnostic criteria in different parts of the world took diverging paths according to regional practice patterns. The main subject of disagreement among the guidelines is the definition of “washout”. The western criteria, including the Liver Imaging Reporting and Data System (LI-RADS), the main diagnostic algorithm for HCC endorsed by the AASLD, choose to restrict the timing of washout to only the portal venous phase (PVP) on HBA-MRI. However, the KLCA-NCC criteria expand the timing to the transitional phase and hepatobiliary phase (HBP) in addition to the PVP. These choices reflect different treatment strategies for HCC.4 In western countries, liver transplantation, particularly from deceased donors, is the primary option, requiring specific diagnosis to maximize organ utilization. By contrast, eastern countries prefer surgical resection and image-guided ablation whose outcomes are closely related to the early detection of HCC. Therefore, Asian countries, including Korea, tend to outweigh sensitive diagnosis over specific diagnosis. In addition to the controversy over washout, other differences among the diagnostic criteria should be noted. LI-RADS includes two additional major imaging features other than washout, threshold growth, and enhancing capsule, which are not evaluated in the KLCA-NCC 2018 and EASL criteria. Unlike the EASL criteria, which consider only hemodynamic imaging features, even on MRI, the LI-RADS and KLCA-NCC 2018 criteria take ancillary

Abbreviations:
AASLD, American Association for the Study of Liver Disease; EASL, the European Association for the Study of the Liver; ECA, extracellular contrast agent; HBA, hepatobiliary contrast agent; HBP, hepatobiliary phase; HCC, hepatocellular carcinoma; KLCA-NCC, the Korea Liver Cancer Association-National Cancer Center; LI-RADS, the Liver Imaging Reporting and Data System; LR-5, LI-RADS category 5; MRI, magnetic resonance imaging; PVP, portal venous phase

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imaging features into account to exclude typical benign lesions or other malignancy.

In this issue of *Clinical and Molecular Hepatology*, Lee and colleagues\(^5\) compare the diagnostic performance of LI-RADS 2018 and KLCA-NCC 2018 criteria in 273 treatment-naïve patients at high risk for HCC. Two hundred and two patients underwent HBA-MRI and 71 patients were evaluated with ECA-MRI. The authors should be congratulated for a timely and relevant comparison between HBA and ECA with application of the latest major diagnostic criteria. The authors properly frame the question regarding the impact of the diagnostic criteria on sensitivity and specificity according to the choice of contrast agent.

Lee et al.\(^5\) showed that on HBA-MRI, the KLCA-NCC 2018 criteria showed significantly better sensitivity for definite HCC than did LI-RADS category 5 (LR-5; 79.1% vs. 68.2%, \(P<0.001\)), without a significant compromise in specificity. The difference in sensitivity between the two diagnostic criteria was more prominent for lesions smaller than 20 mm (KLCA-NCC vs. LI-RADS, 75.8% vs. 51.6%). The improved sensitivity obtained by extending the washout to the HBP with the KLCA-NCC 2018 criteria is consistent with previous work,\(^6,7\) as the advantages of HBA-MRI mainly come from capturing the HBP signal intensity changes preceding the typical vascular profile changes during hepatic carcinogenesis. As HBA-MRI presents some drawbacks for arterial phase imaging, the current stringent washout criteria for LR-5 probably fail to obtain acceptable sensitivity. These strict washout criteria do not seem consistent with the main cause of the recent enthusiasm for HBA-enhanced sensitivity. This rather confusing approach toward washout timing in the western criteria is probably owing to a concern over a loss of specificity.

Lee et al.\(^5\) found that the specificity for definite HCC according to the KLCA-NCC 2018 criteria (93.9%) with washout criteria extended to the HBP was not significantly different to that of LR-5 (95.4%) with washout restricted to the PVP only. This high specificity of the KLCA-NCC 2018 criteria can be achieved by considering ancillary imaging features such as marked T2 hyperintensity or a targetoid appearance to exclude common causes of false-positive cases such as hemangiomas or non-HCC malignancy. When washout timing was expanded, studies taking such ancillary imaging features into account successfully maintained high specificity (84.2–87.4%),\(^6,7\) while studies not considering them reported low specificity (48.4–68.1%).\(^8,9\) However, it should be noted that the reported specificity in the study of Lee et al.\(^5\) was higher than that in previous studies. Their study may overestimate specificity as it only included surgically-proven malignancy. For example, a non-HCC malignancy miscategorized as HCC, especially with the KLCA-NCC 2018 criteria, and then treated with ablation therapy could have been excluded from the final cohort of this study. Some frequently encountered benign lesions such as eosinophilic infiltration were not listed as false-positive lesions in this study.

This study\(^5\) claimed that on ECA-MRI, the sensitivities of LR-5 and definite HCC in the KLCA-NCC 2018 criteria did not significantly differ. Nevertheless, the sensitivity of 75.8% for LR-5 was still higher than the value of 69.4% for definite HCC in the KLCA-NCC 2018 criteria, although statistically not significant. This higher sensitivity for LR-5 can be attributed to the additional major imaging features that are addressed in LI-RADS 2018 but not in KLCA-NCC 2018. Lee et al.\(^5\) found that two HCCs were diagnosed as LR-5 because of threshold growth, while these were not regarded as definite HCC according to KLCA-NCC 2018. As Lee et al.\(^5\) included only 62 HCCs evaluated with ECA, the study might be underpowered in respect to detection of the sensitivity difference between the two diagnostic criteria. The exclusion of threshold growth and enhancing capsule from the major features for the diagnosis of definite HCC in the KLCA-NCC 2018 criteria may likely mitigate their sensitivity on ECA-MRI.

MRI contrast agents need to be selected with careful consideration of the available treatment options and liver function of patients; appropriate diagnostic criteria should be applied to maximize the unique advantages of each MRI contrast agent. When surgical resection and image-guided ablation are considered as curative options, HBA-MRI comes into its own, presenting optimal image quality in patients with preserved liver function, and survival benefits that were demonstrated in a recent large-scale study.\(^10\) Under such circumstances, diagnostic criteria should pursue sensitivity at the expense of specificity, which is a tenet of the KLCA-NCC 2018 criteria. To hold the specificity loss within an acceptable range with expanded washout timing, ancillary imaging features excluding common false positives should be incorporated. Contrarily, in a clinical setting where liver transplantation from deceased donors is considered as a primary option, ECA-MRI provides excellent specificity and image quality is not degraded by poor liver function. When interpreting ECA-MRI, LI-RADS criteria considering threshold growth and capsular enhancement along with washout as a major diagnostic criterion may ameliorate sensitivity. The next version of the KLCA-NCC criteria is anticipated to consider more detailed features for ECA-MRI.
Conflicts of Interest

The author has no conflicts of interests to disclose.

REFERENCES

Is tenofovir and entecavir combination therapy still the optimal treatment for chronic hepatitis B patients with prior suboptimal response?

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**Keywords:** Chronic hepatitis B; Entecavir; Suboptimal response; Tenofovir

It is well-known that consistently high hepatitis B virus (HBV) DNA levels increase the risk of fibrosis progression and hepatocellular carcinoma (HCC) development in patients with chronic hepatitis B (CHB). Therefore, the primary goal of CHB treatment is complete suppression of HBV DNA by antiviral treatment. Before the introduction of nucleos(t)ide analogues (NAs) with a high genetic barrier, such as entecavir (ETV) and tenofovir disoproxil fumarate (TDF), long-term treatment of NAs with a low genetic barrier, such as lamivudine (LAM), adefovir dipivoxil (ADV) and telbivudine (LdT), led to drug resistance in many CHB patients. Until the introduction of ETV or TDF, the standard treatment was to add ADV to LAM or LdT for the treatment of CHB patients with LAM or LdT resistance. However, CHB patients receiving LAM or LdT/ADV combination therapy often show a suboptimal response. In patients with this suboptimal response, it is known that the risk of developing resistance to various NAs increases, as well as the risk of developing end-stage liver disease and HCC. Therefore, the current guidelines recommended that HBV DNA maintain serum HBV DNA level below the detection limit of real-time polymerase chain reaction as the primary treatment goal. In the past, a combination of ETV-based regimens had been attempted to treat the CHB patients who had suboptimal response to LAM or LdT/ADV combination therapy. However, since the introduction of TDF with strong antiviral efficacy in the treatment of CHB, TDF monotherapy or TDF+other NAs regimens have been attempted to treat patients with various drug resistance. Berg et al. conducted a prospective randomized controlled trial to compare the antiviral effects of TDF monotherapy and TDF+emtricitabine (FTC) combination therapy in patients with suboptimal responses to ADV. According to the results of their study, complete virologic response (CVR) of TDF monotherapy and TDF+FTC combination therapy groups was 81% and 81%, respectively, at 48 weeks; and there was no difference between the two groups. In a retrospective study of CHB patients with suboptimal response to ADV with or without NAs in LAM-resistant CHB, Cho et al. showed that the CVR of TDF monotherapy group and TDF with NA combination group at 48 weeks was 81.8% and 85.9%, respectively; and...
there was also no significant difference between the two groups ($P=0.075$). In a small randomized controlled trial, Lee et al. compared the antiviral efficacy between switching to TDF+NA therapy and continuing current ADV+NA therapy in patients with suboptimal response to ADV-based therapy. The results of their study showed that TDF+NA therapy was significantly higher in CVR compared to continued ADV+NA therapy ($87.5\%$ vs. $37.5\%$ at 48 weeks, $P=0.002$). In addition, TDF showed good effects on CHB patients with ETV, ADV resistance, and multidrug resistance, as well as LAM resistance. Lim et al. compared the effects of TDF monotherapy versus TDF and ETV combination therapy in patients with ETV-resistant CHB with multiple drug failure in a randomized controlled trial, and the CVR of TDF and TDF+ETV groups was $71\%$ and $73\%$, respectively, at 48 weeks. There was no difference between the two groups ($P=0.99$). In a study comparing the effects of TDF monotherapy versus TDF and ETV combination therapy in ADV-resistant CHB patients with multiple drug failure, the CVR at 48 weeks was $62\%$ and $63.5\%$ for TDF and TDF/ETV groups, respectively; and there was also no significant difference between the two groups ($P=0.88$). Moreover, the results of several different studies showed no difference in CVR between TDF monotherapy and TDF+ETV combination therapy, the combination regimen of the most potent NAs to date, in CHB patients who are resistant to various NAs (Table 1).

Woo et al. conducted a prospective randomized controlled study to compare the effectiveness and efficacy between TDF+ETV therapy and LAM/LdT+ADV maintenance therapy for LAM-resistant patients who had suboptimal responses to LAM+ADV combination therapy. As predicted from the results of many previous clinical studies, the results of this study showed that TDF+ETV group had a significantly higher CVR at 48 weeks compared to LAM/LdT+ADV maintenance therapy group ($93.33\%$ vs. $6.52\%$, $P<0.001$). The clinical significance of this study was to confirm the expected results based on the results of previous retrospective studies through prospective trials. However, based on the results of several studies, including the studies conducted by Lim et al., TDF monotherapy alone was expected to have a sufficient effect even in patients with suboptimal responses to LAM+ADV. Therefore, this study had a significant limitation of not comparing the effect of TDF monotherapy to that of TDF+ETV group. In real clinical practice, most patients with LAM+ADV suboptimal responses have already been treated with either TDF monotherapy or TDF+NA combination therapy. Therefore, the results of this study may be considered a little late to have practical significance in clinical practice. Recently, patients who participated in the two aforementioned studies conducted by Lim et al. reported the results of treatment for 244 weeks after switching from TDF+ETV to TDF monotherapy at 48 weeks. The CVR in ETV and ADV resistance groups increased to $84.4\%$ and $73.5\%$, respectively, at 240 weeks; and there was no significant difference between the two groups ($P=0.07$). However, eGFR and bone mineral density were significantly decreased at the 240-week time point compared to the baseline ($P<0.001$), reflecting the concern about the safety of long-term use of TDF. Due to these adverse effects of TDF, tenofovir alafenamide (TAF) has been recommended recently, especially in patients who are at risk

### Table 1. Summary of anti-viral efficacy results of tenofovir-based therapy in patients with suboptimal response or resistance to NAs

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Study population</th>
<th>Intervention</th>
<th>Primary efficacy end point</th>
<th>Virologic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berg et al. (2010)</td>
<td>Prospective</td>
<td>Suboptimal response to ADV</td>
<td>TDF (n=53) vs. TDF/FTC (n=52)</td>
<td>HBV DNA level &lt;400 copies/mL at 48 weeks</td>
<td>81% vs. 81% (P=NS)</td>
</tr>
<tr>
<td>Cho et al. (2015)</td>
<td>Retrospective</td>
<td>Suboptimal response to ADV with or without NAs in LAM-resistant</td>
<td>TDF (n=18) vs. TDF/NA (n=107)</td>
<td>HBV DNA level &lt;20 IU/mL at 48 weeks</td>
<td>81.8% vs. 85.9% (P=0.750)</td>
</tr>
<tr>
<td>Lee et al. (2016)</td>
<td>Prospective</td>
<td>Partial responses to ADV+NA therapy for NA resistance</td>
<td>TDF/NA (n=16) vs. continued ADV/NA (n=16)</td>
<td>HBV DNA level &lt;60 IU/mL at 48 weeks</td>
<td>81.3% vs. 56.3% (P&lt;0.001)</td>
</tr>
<tr>
<td>Lim et al. (2016)</td>
<td>Prospective</td>
<td>ETV-resistant</td>
<td>TDF (n=45) vs. TDF/ETV (n=45)</td>
<td>HBV DNA level &lt;15 IU/mL at 48 weeks</td>
<td>71% vs. 73% (P=0.99)</td>
</tr>
<tr>
<td>Lim et al. (2016)</td>
<td>Prospective</td>
<td>ADV-resistant</td>
<td>TDF (n=50) vs. TDF/ETV (n=52)</td>
<td>HBV DNA level &lt;15 IU/mL at 48 weeks</td>
<td>62% vs. 63.5% (P=0.88)</td>
</tr>
</tbody>
</table>

NA, nucleos(t)ide analogue; ADV, adefovir dipivoxil; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; HBV, hepatitis B virus; NS, not significant; LAM, lamivudine; ETV, entecavir.
of developing renal or bone disease.\textsuperscript{3,5} Therefore, in the future, clinical studies are needed to verify the effectiveness of TAF-based regimens in patients with CHB who are resistant or suboptimal to various NAs.

**Conflicts of Interest**

The author has no conflicts of interests to disclose.

**REFERENCES**

Low-level viremia in patients undergoing antiviral therapy: Does it indicate time for a change?

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Keywords: Hepatitis B; Medication adherence; Hepatocellular carcinoma; Liver cirrhosis

The goal of chronic hepatitis B (CHB) treatment is to decrease liver disease-related mortality by preventing fibrosis progression and development of hepatocellular carcinoma (HCC). Serum hepatitis B virus (HBV) DNA testing provides a direct measure of the level of viral replication and is a strong predictor of disease progression and long-term outcomes in CHB. Nucleos(t)ide analogs (NAs) are available to effectively inhibit HBV replication. When serum HBV DNA levels decrease to undetectable levels in a real-time polymerase chain reaction assay by NAs use, it is defined as a virologic response (VR).

VR can be achieved in most patients undergoing therapy with potent NAs. However, some patients show persistent or intermittent episodes of detectable, but low levels of serum HBV DNA (<2,000 IU/mL), referred to as low-level viremia (LLV) or suboptimal VR. When using low genetic barrier NAs such as lamivudine, LLV signals emerging resistance and virologic breakthrough. Continued use of high genetic barrier NAs in patients with LLV can further induce VR with very low risk of resistance and virologic breakthrough. Thus, it is unclear whether LLV in patients under high genetic barrier NAs means a time for a change as in patients under low genetic barrier NAs. In this issue, Lee et al. analyzed the association between LLV and long-term outcomes in 894 patients with CHB who were treated with entecavir, a high genetic barrier NA, to address this issue.

The goal of NA treatment is to achieve persistent undetectable serum HBV DNA levels, known as maintained virologic response (MVR). However, virologic tools used to measure HBV DNA have improved over the past years. MVR that would have been previously defined using old assays could now be classified as LLV with the use of more sensitive assays. Patients reaching LLV, rather than having undetectable HBV DNA levels, may be sufficient for improving patient prognosis. The study by Lee et al. supports that continued treatment with high genetic barrier NAs is suffi-
cient in adherent patients with LLV. In this study, LLV was associated with HCC in the entire cohort, indicating that LLV is an alarming sign of poor prognosis. However, there was no association between LLV and poor prognosis when the analysis was limited to 617 adherent patients. Thus, poor prognosis in patients with LLV was mostly driven by poor adherence. Hence, the study suggested that LLV means time to check adherence but is not a time for a change in treatment when using a high genetic barrier NA. This study provides evidences on how to manage patients with LLV when using high genetic barrier NA.

However, the study design was an observational cohort study with inherent limitations. Lee et al.8 provide excellent discussion on the potential limitations and implications of their findings. In addition, some points need to be further discussed. LLV usually refers to a subgroup of patients that exhibit persistent or intermittent episodes of detectable, but low serum HBV DNA levels (<2,000 IU/mL) without virologic breakthrough. However, patients with virologic breakthrough were included in this study. Out of the 240 patients with LLV, 56 (23.3%) switched to tenofovir and were censored when entecavir treatment was switched to tenofovir. In contrast to findings from Lee et al.,8 some studies suggest that changing instead of continuing with the current treatment may be better approach. In an analysis of 239 patients with paired liver biopsy, LLV was more frequently observed for patients with fibrosis progression (50%) than in patients with fibrosis regression (19%) or indeterminate fibrosis (26%) (P = 0.015), suggesting that LLV may still promote fibrosis progression.10 In our previous study, we observed a higher risk of HCC in cirrhotic patients with LLV than with MVR.5 In a randomized trial conducted in Korea, patients with CHB with detectable HBV DNA (≥60 IU/mL) treated with 0.5 mg of entecavir for >12 months showed higher VR (HBV DNA <20 IU/mL) after switched to tenofovir (55%) than in patients that continued with entecavir (20%, P = 0.022).11

Therefore, here is the question: Is LLV during high genetic barrier NA therapy a meaningful sign to change therapy? In this study, Lee et al.8 showed similar risks of liver-related death, transplantation, HCC, and hepatic decompensation between MVR and LLV groups in good adherent patients. However, the ultimate question is whether a change in therapy (switch to another NA or adding an additional NA) can decrease the risk of liver-related mortality or HCC among patients showing LLV. To our knowledge, no information is available on whether changing NA therapy can decrease the risk of liver-related mortality or HCC compared to continuing same treatment. In the recently revised guidelines of the Korean Association for the Study of the Liver, either continued treatment or switching to another NA has been suggested as a treatment option. The findings from Lee et al.,8 provide another clue for the answer, yet, are imperfect to direct one approach. Until more robust data are available, the decision to continue, switch, or add another NA should be made based on available evidence, precise follow-up, and careful assessment of risks and benefits.

Authors’ contribution
Manuscript writing: Jung Hee Kim and Dong Hyun Sinn
Final approval of manuscript: Jung Hee Kim and Dong Hyun Sinn

Conflicts of Interest
The authors have no conflicts of interests to disclose.

REFERENCES

Clinical application of ultrasonography-guided percutaneous liver biopsy and its safety over 18 years

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\(^1\)Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul; \(^2\)Department of Internal Medicine, Soonchunhyang University College of Medicine, Bucheon; \(^3\)Department of Biostatistics, Chung-Ang University Graduate School, Seoul; \(^4\)Department of Pathology, Soonchunhyang University College of Medicine, Bucheon, Korea

Abbreviations:
- CT, computed tomography; DILI, drug-induced liver disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cholangitis

*Y Chang and Ji Kim contributed equally as co-first authors.

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INTRODUCTION

Percutaneous liver biopsy was first introduced in 1883. Since then, it has been the gold standard for the evaluation of liver disease. According to the American Association for the Study of Liver Disease guidelines, liver biopsy is an essential diagnostic tool for the diagnosis of parenchymal liver disease, and the evaluation of inflammation and fevers of unknown origin. However, liver biopsy is an invasive procedure associated with several complications. The most common complications were abdominal pain, which was reported in almost 30% of the patients, followed by bleeding (0.3%) and death (0.03%), respectively. However, these complication rates have been reported mainly in blind liver biopsies, and the complication rate is expected to decrease significantly under ultrasonography-guided liver biopsies. Currently, image-guided liver biopsies have replaced traditional blind liver biopsies. It is widely used to maximize the effectiveness of the procedure and minimize the complication rate.

Recently, many noninvasive methods have been developed to replace liver biopsies. The most widely used noninvasive test is transient elastography. It has been used since 2005 to stage liver fibrosis and steatosis. Two-dimensional shear wave elastography can be used to determine the fibrosis stage of the liver based on the concurrent real-time grayscale. Moreover, magnetic resonance electrography can also be used to evaluate the entire liver fibrosis stage and is widely used to replace liver biopsies in clinical trials, especially phase II trials. Therefore, the role of a traditional liver biopsy is expected to change significantly in the immediate future.

In this study, we evaluated the changes in liver biopsy indications over the past 18 years and the safety of ultrasonography-guided liver biopsies in the era of noninvasive assessments of liver fibrosis.
the cause and severity of the patient’s liver disease. During this period, five patients underwent blind biopsies and 97 patients underwent computed tomography (CT)-guided biopsies. The 102 patients who underwent blind or CT-guided liver biopsies were excluded from the study. The final number of eligible patients included in the study was 1,944. The clinical indications for liver biopsy, the suspected diagnosis before the biopsy, and the final diagnosis after the biopsy were collected. The medications taken at the time of biopsy and complications after the biopsy were also reviewed. Liver cirrhosis was assessed by ultrasound, defined as the presence of a nodular liver surface, round edges, and hypoechoic nodules in the liver parenchyma, which represent regenerative nodules.\textsuperscript{11}

The study protocol was approved by the Institutional Review Board of SoonChunHyang University Bucheon Hospital (IRB number SCHBC 2019-10-022). The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki.

Liver biopsy

Ultrasonography-guided liver biopsies were performed by four expert hepatologists experienced with more than 1,000 ultrasound procedures and over 200 liver biopsies. The patients were admitted to the hospital the day before the procedure and fasted for at least 12 hours. Prior to the procedure, routine laboratory tests, including coagulation parameters, chest X-rays, and abdominal ultrasounds were performed. During the procedure, a transthoracic approach was routinely used with the patient in the supine position. Local anesthesia using a 2% lidocaine solution was administered, and the biopsy was performed using an 18-gauge Tru-cut needle. After the liver biopsy, the standard compressive dressing was immediately applied to the wound and bed rest was advised for more than 6 hours for hemostasis. The patients remained under observation for one day following the procedure. The biopsy specimens were fixed in formalin and embedded in paraffin. Each biopsy specimen was analyzed by three experienced hospital pathologists. The histologic grading and staging of chronic hepatitis were performed based on standardized guidelines proposed by the Korean Study Group for the Pathology of Digestive Diseases.\textsuperscript{12}

### Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Total (n=1,944)</th>
<th>Before 2008 (n=456)</th>
<th>2008 to 2012 (n=859)</th>
<th>After 2013 (n=629)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48 (37–57)</td>
<td>47 (35–57)</td>
<td>46 (37–54)</td>
<td>51 (41–60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males</td>
<td>1,135 (58.4)</td>
<td>336 (73.7)</td>
<td>481 (56.0)</td>
<td>318 (50.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.05±1.97</td>
<td>13.26±2.02</td>
<td>13.19±1.88</td>
<td>12.7±2.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (10\textsuperscript{11}/µL)</td>
<td>202.5±85.74</td>
<td>193.78±85.23</td>
<td>201.56±87.13</td>
<td>210.1±83.64</td>
<td>0.007</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.82 (0.59–1.26)</td>
<td>0.78 (0.55–1.19)</td>
<td>0.85 (0.62–1.27)</td>
<td>0.82 (0.6–1.31)</td>
<td>0.011</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.92±0.56</td>
<td>3.93±0.53</td>
<td>4.01±0.54</td>
<td>3.79±0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>51 (31–94)</td>
<td>52 (31–98)</td>
<td>48 (30–91)</td>
<td>52 (32–95)</td>
<td>0.195</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>48 (24–110)</td>
<td>45 (25–93.5)</td>
<td>47 (24–108.5)</td>
<td>50 (24–118.75)</td>
<td>0.701</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.02±0.97</td>
<td>1.05±0.83</td>
<td>1.00±0.89</td>
<td>1.02±1.15</td>
<td>0.576</td>
</tr>
<tr>
<td>PT (INR)</td>
<td>1.08±0.16</td>
<td>1.09±0.14</td>
<td>1.08±0.19</td>
<td>1.09±0.15</td>
<td>0.560</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>563 (28.96)</td>
<td>141 (30.92)</td>
<td>244 (28.41)</td>
<td>178 (28.30)</td>
<td>0.567</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>56 (2.88)</td>
<td>9 (1.97)</td>
<td>32 (3.73)</td>
<td>15 (2.38)</td>
<td>0.129</td>
</tr>
<tr>
<td>Warfarin</td>
<td>2 (0.10)</td>
<td>1 (0.22)</td>
<td>0 (0.00)</td>
<td>1 (0.16)</td>
<td>0.432</td>
</tr>
<tr>
<td>NSAID</td>
<td>73 (3.76)</td>
<td>16 (3.51)</td>
<td>35 (4.07)</td>
<td>22 (3.50)</td>
<td>0.803</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation, median (range) for continuous variables, or frequency (percentage) for categorical variables. The proportions are presented as percentages for categorical variables.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; INR, international normalized ratio; NSAID, non-steroidal anti-inflammatory drugs.
Statistical analysis

Frequencies and percentages were used for the descriptive statistics. Statistical differences between the groups were investigated using the chi-squared test and Student’s t-test for the comparison of two independent groups and ANOVA tests were used for groups of three or more. Spearman’s analysis was used to investigate the correlations between the variables. All statistical analyses were performed using R (version 3.3.3; The R Foundation for Statistical Computing, Vienna, Austria) and SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA). Statistical significance was defined at P<0.05.

RESULTS

Baseline characteristics of the study population

Table 1 presents the baseline characteristics of the study population according to the year of the liver biopsy. A total of 1,944 patients underwent liver biopsies over the last 18 years. The median age of the study population was 48 years and 58% was male. The median age of the study population and the proportion of females tended to increase significantly over time. Despite advising patients to abstain from antiplatelet agents or anticoagulants, nine patients (1.97%) continued taking antiplatelet agents, and one patient was undergoing warfarin treatment. These patients underwent biopsies after discontinuing the drug for 3 days after hospitalization.

Changes in liver biopsy indications over time

In all 1994 patients, hepatitis B virus (HBV) was the most common indication for a liver biopsy (25.3%), followed by suspected malignancy (20.5%), nonalcoholic fatty liver disease (NAFLD) (13.0%), drug-induced liver disease (DILI) (11.7%), and alcohol-related liver disease (8.2%) during the overall study period (Fig. 1). The top three major indications for a liver biopsy were consistently chronic hepatitis B, malignancy, and NAFLD, but the priority of the indications changed over time (Table 2). Before 2008, malignancy (34.6%) and chronic HBV (26.5%) accounted for more than 50% of the liver biopsy indications. From 2008 to 2012, the most common indication was chronic HBV (31.3%), followed by malignancy (15.7%) and NAFLD (12.6%). In the 5 years since 2013, the proportion of each of the three main indications was similar, with NAFLD accounting for the highest proportion, at 17.2%.

Figure 2 shows that liver biopsies for chronic hepatitis B and C increased by 2012, accounting for 40.3% of the total. In the last 5 years since 2013, the proportion of viral hepatitis decreased substantially to 18.9%. Liver biopsies to distinguish malignancies
also declined since 2008, from 34.6% to 16.7%. In contrast, liver biopsies for NAFLD and DILI increased gradually from 8.1% to 17.2% and 5.5% to 14.9%, respectively, since 2008. The proportion of biopsies for alcohol-related liver disease and autoimmune hepatitis/primary biliary cholangitis (PBC) did not change until 2012. However, since 2013, they have increased sharply from 5.9% to 12.2% and 5.4% to 13.8%, respectively.

**Table 2.** Trends in the indications for liver biopsy over time

<table>
<thead>
<tr>
<th></th>
<th>Total (n=1,944)</th>
<th>Before 2008 (n=456)</th>
<th>2008 to 2012 (n=859)</th>
<th>After 2013 (n=629)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>492 (25.3)</td>
<td>121 (26.5)</td>
<td>269 (31.3)</td>
<td>102 (16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malignancy</td>
<td>398 (20.5)</td>
<td>158 (34.6)</td>
<td>135 (15.7)</td>
<td>105 (16.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAFLD</td>
<td>253 (13.0)</td>
<td>37 (8.1)</td>
<td>108 (12.6)</td>
<td>108 (17.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drug-induced liver disease</td>
<td>228 (11.7)</td>
<td>25 (5.5)</td>
<td>109 (12.7)</td>
<td>94 (14.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol-related liver disease</td>
<td>160 (8.2)</td>
<td>32 (7.0)</td>
<td>51 (5.9)</td>
<td>77 (12.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Autoimmune hepatitis or PBC</td>
<td>158 (8.1)</td>
<td>25 (5.5)</td>
<td>46 (5.4)</td>
<td>87 (13.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV</td>
<td>114 (5.9)</td>
<td>20 (4.4)</td>
<td>77 (9.0)</td>
<td>17 (2.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>107 (5.5)</td>
<td>27 (5.9)</td>
<td>54 (6.3)</td>
<td>26 (4.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Etiology of liver cirrhosis</td>
<td>34 (1.7)</td>
<td>11 (2.4)</td>
<td>10 (1.2)</td>
<td>13 (2.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

HBV, hepatitis B virus; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cholangitis; HCV, hepatitis C virus.

**Discordances in diagnoses before and after liver biopsies**

Table 3 shows the diagnostic agreement before and after biopsy. There were 51 cases (2.6%) of discordance between the suspected diagnosis and the final diagnosis. The 51 discordant cases included 19 cases of suspected malignancies and 17 cases of suspected DILI. Of the 19 patients with suspected malignancies, 10
were finally diagnosed as benign masses, such as hemangioma (52.6%), and the remaining nine were diagnosed with other diseases. Of the 17 patients suspected with DILI, 11 (64.7%) were finally diagnosed as autoimmune hepatitis, PBC or overlap syndrome, and five (29.4%) as NAFLD. However, eight patients suspected of autoimmune hepatitis or PBC proved to be NAFLD or DILI after the liver biopsies.

In addition, there were two noticeable discordant cases. A 58-year-old male was suspected to have liver cirrhosis but it was difficult to identify whether the cause of the cirrhosis was HBV or alcohol by serological and radiological testing. However, after the liver biopsy, the pathologic analysis indicated that the main cause of the chronic liver injury was alcohol rather than HBV. Valuable information was obtained from the liver biopsy, and without the liver biopsy, it would not have been possible to accurately diagnose the patient using only his history or laboratory results. The second noticeable discordant case was a 48-year-old female diagnosed with a benign mass, although the suspected diagnosis was focal fat-sparing before the liver biopsy. She had a hypoechoic lesion in the background of fatty liver by ultrasonography and was suspected to have a fatty liver with a focal fat-sparing zone. Additional CT and magnetic resonance imaging (MRI) tests could not differentiate between the focal fat-sparing zone vs. the benign mass in aspects such as focal nodular hyperplasia. A targeted liver biopsy was performed for the differential diagnosis of the focal lesion and it was finally confirmed as a hemangioma.

### Liver biopsy in cirrhosis

Among the 1,944 patients included in the study population, 563 (30.0%) had liver cirrhosis at the time of their liver biopsy. In these patients, a liver biopsy was conducted to determine the etiology of the liver cirrhosis and accurately determine the degree of liver fibrosis. Most of the pre-biopsy diagnoses were consistent with the final diagnoses, including cryptogenic liver cirrhosis. However, in contrast, the histological results of these patients revealed that 16% (90 of 563) had no liver cirrhosis. The histological results of the patients diagnosed with cirrhosis by ultrasound

---

**Table 3. Diagnostic concordance before and after biopsy**

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>HBV</th>
<th>HCV</th>
<th>Alcohol</th>
<th>NAFLD</th>
<th>AI/PBC</th>
<th>DILI</th>
<th>Malignancy</th>
<th>LC</th>
<th>Etc.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>35</td>
<td>36</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>492</td>
</tr>
<tr>
<td>HBV</td>
<td>491</td>
<td></td>
<td>3</td>
<td>494</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>113</td>
<td></td>
<td></td>
<td>113</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alcohol</td>
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<td>160</td>
<td>1</td>
<td>1</td>
<td>163</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAFLD</td>
<td>245</td>
<td></td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>254</td>
<td></td>
<td></td>
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<tr>
<td>Autoimmune hepatitis</td>
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<td>56</td>
<td>7</td>
<td>2</td>
<td>66</td>
<td></td>
<td></td>
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<tr>
<td>PBC</td>
<td>55</td>
<td></td>
<td>2</td>
<td>1</td>
<td>58</td>
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<td>Overlap syndrome</td>
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<td>26</td>
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<tr>
<td>DILI</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>200</td>
<td>1</td>
<td>2</td>
<td>210</td>
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<td>53</td>
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<tr>
<td>Other cancer</td>
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<td>41</td>
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<td>Benign mass</td>
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<td></td>
<td>10</td>
<td>4</td>
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<td></td>
<td></td>
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<td>Cryptogenic LC</td>
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<td>32</td>
<td>2</td>
<td>38</td>
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<td>3</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>6</td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>15</td>
<td>11</td>
<td>8</td>
<td>51</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>492</td>
<td>114</td>
<td>160</td>
<td>253</td>
<td>158</td>
<td>228</td>
<td>398</td>
<td>34</td>
<td>107</td>
<td>1,944</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; AI, autoimmune hepatitis; PBC, primary biliary cholangitis; DILI, drug-induced liver injury; LC, liver cirrhosis; HCC, hepatocellular carcinoma; IHCC, intrahepatic cholangiocarcinoma.
were: F1 (nine patients, 1.60%), F2 (six patients, 1.07%), F3 (75 patients, 13.32%), and F4 (473 patients, 84.01%). As shown in Table 4, the patients with cirrhosis were significantly older than those without cirrhosis (median age of 53 years vs. 45 years) and comprised a higher proportion of males (66.1% vs. 55.2%). Among the cirrhotic patients, 20.2% of the patients showed ascites. Four hundred thirty-nine patients (78.0%) were Child-Pugh class A and 124 (20.7%) were Child-Pugh class B or C. The median model for end-stage liver disease (MELD) score was 8.7. The presence of cirrhosis did not affect the frequency of complications (P=0.289).

Complications after liver biopsy

Painkillers were prescribed during hospitalization for 116 patients (6.0%) because of pain and there was no record of hypotension or pneumothorax. However, this was a retrospective study and thus, these minor complications may have been underestimated.

The major complication rate was 0.05%. A single major complication associated with a liver biopsy occurred during the study period. The patient was a 61-year-old female who had cryptogenic liver cirrhosis. She had thrombocytopenia (71,000/µL) and prolonged prothrombin time (15.5 seconds). Her baseline Child-Pugh score was 6, and her MELD score was 13. The liver biopsy was done without immediate complications and the patient was discharged without procedure-related complications by abdominal ultrasonography. However, four days post-liver biopsy, the patient was re-admitted due to abdominal pain and delayed bleeding from the biopsy site was found. The patient’s vital signs and laboratory findings, including hemoglobin, were stable and no procedure was required for hemostasis. The patient was discharged successfully after supportive care for 5 days.

Table 4. Baseline characteristics of the study population with or without a diagnosis of liver cirrhosis

<table>
<thead>
<tr>
<th>Total (n=1,944)</th>
<th>Liver cirrhosis (-) (n=1,382)</th>
<th>Liver cirrhosis (+) (n=562)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 (37–57)</td>
<td>45 (34–55)</td>
<td>53 (45–61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1,135 (58.35)</td>
<td>763 (55.23)</td>
<td>372 (66.07)</td>
<td></td>
</tr>
<tr>
<td>WBC (10^3/µL)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5,700 (4,500–7,040)</td>
<td>5,995 (4,800–7,300)</td>
<td>5,070 (3,905–6,500)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.05±1.97</td>
<td>13.29±1.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (10^3/µL)</td>
<td>200 (146–251)</td>
<td>220 (175–263)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.82 (0.59–1.26)</td>
<td>0.76 (0.56–1.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.92±0.56</td>
<td>4.03±0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>51 (31–94)</td>
<td>51 (29–100)</td>
<td>0.537</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>48 (24–110)</td>
<td>58 (26–147)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.02±0.97</td>
<td>1.02±0.97</td>
<td>0.820</td>
</tr>
<tr>
<td>PT (INR)</td>
<td>1.08±0.16</td>
<td>1.05±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>None</td>
<td>1,784 (91.72)</td>
<td>1,335 (96.6)</td>
<td>449 (79.75)</td>
</tr>
<tr>
<td>Mild</td>
<td>137 (7.04)</td>
<td>43 (3.11)</td>
<td>94 (16.7)</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>24 (1.23)</td>
<td>4 (0.29)</td>
<td>20 (3.55)</td>
</tr>
<tr>
<td>Child-Pugh class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class A</td>
<td>N/A</td>
<td>N/A</td>
<td>413 (73.36)</td>
</tr>
<tr>
<td>Class B or C</td>
<td>N/A</td>
<td>N/A</td>
<td>150 (26.64)</td>
</tr>
<tr>
<td>MELD</td>
<td>N/A</td>
<td>N/A</td>
<td>8.75 (7.6–10.59)</td>
</tr>
<tr>
<td>Complication</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation, median (range) for continuous variables, or frequency (percentage) for categorical variables. The proportions are presented as percentages for categorical variables.

WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; INR, international normalized ratio; N/A, not applicable; MELD, model for end-stage liver disease.
DISCUSSION

In this study, we retrospectively analyzed all of the cases of ultrasonography-guided percutaneous liver biopsies for the past 18 years. Currently, percutaneous liver biopsy under ultrasonography guidance has been the most generally used method. Only when there were specific reasons, such as high bleeding tendency or large amounts of perihepatic ascites, were transjugular or CT-guided liver biopsies performed instead of ultrasonography-guided percutaneous liver biopsies. We included only patients who underwent ultrasonography-guided percutaneous liver biopsies to exclude those extreme cases and analyze a homogenous and standardized liver biopsy study population. The number of liver biopsies decreased from 2013 to 2018 and the indications for liver biopsies also changed by the period. The most common indication for liver biopsy during the entire study period was chronic HBV, followed by malignancy, and NAFLD. However, since 2013, the rate of NAFLD has increased rapidly, making it the most common indication. The rate of liver biopsies for chronic HBV and malignancy has declined, whereas the incidence of DILI, alcohol-related liver disease, and autoimmune hepatitis/PBC has increased sharply in the recent 5 years. Overall, a major adverse event of delayed bleeding at the biopsy site was identified, which was resolved by conservative management.

The decrease in liver biopsies for viral hepatitis was mainly attributed to the development of noninvasive methods for the assessment of liver fibrosis; the introduction of potent antiviral agents, such as entecavir, tenofovir, and direct-acting agents for hepatitis C; and the expansion of indications for antiviral treatment. In addition, advances in radiologic imaging techniques have reduced the number of liver biopsies for malignancies. Fatty liver has recently emerged as a major etiological factor underlying liver disease, which has rapidly increased the number of liver biopsies for NAFLD. These dynamic changes in indications for liver biopsy showed epidemiological changes over time in the study population.

Liver biopsy has been considered the most specific test to assess liver disease. However, the clinical usefulness and impact of liver biopsies have been underestimated in the recent two decades due to the development of alternative noninvasive tests and new insight into the limitations of liver biopsies. Various types of liver elastography have been developed, with relatively accurate assessments of advanced liver fibrosis. The combination of medical history, serologic and radiologic evaluation, and liver elastography is adequate for the diagnosis of liver disease in most instances. In terms of solid liver lesions, multiphasic contrast-enhanced CT or MRI can discern focal liver lesions in most cases. With the development of imaging techniques, a liver biopsy is rarely needed to distinguish focal liver lesions.

Liver biopsies, however, have their own unique role in the differential diagnosis and management of various liver diseases. A liver biopsy is essential for the diagnosis of autoimmune hepatitis, PBC—especially anti-mitochondrial antibody-negative—and several rare infiltrative diseases, such as amyloidosis and the hepatic involvement of lymphoma. In addition, only liver biopsy can differentiate simple steatosis from steatohepatitis in current clinical practice, which is important in treatment decisions in specific cases of chronic HBV infections. For the diagnosis of malignant tumors including hepatocellular carcinoma (HCC), histological confirmation is the most specific and useful method, especially when imaging findings are ambiguous. In fact, a retrospective analysis of the UNOS/OPTN database reported that 20.9% of the 789 patients who underwent liver transplantation for HCC were finally diagnosed with benign nodules. In our study as well, a considerable discrepancy (2.6%) existed between the suspected diagnosis before liver biopsy and the final diagnosis. These results suggest the clinical importance of liver biopsies despite the development of noninvasive tests. In addition, among 563 patients who had ultrasonographic liver cirrhosis in this study, 16% were revealed not to have histologic liver cirrhosis. The ability to differentially diagnose the presence of liver cirrhosis and assess the exact fibrosis stage, as well as determine the etiology of chronic liver disease, is critical in several situations, especially to establish disease-specific treatments, such as antiviral therapy in viral hepatitis and steroid therapy in alcohol-related liver disease. Liver biopsies complement noninvasive methods, playing a critical role in the evaluation and management of liver fibrosis.

In terms of safety, our study reported a substantially lower rate of major complications (0.05%) than previous reports. In addition, we found that liver biopsies were safe in patients with cirrhosis and/or ascites, contrary to the concerns of clinicians. Some studies have reported that peritoneal ascites did not significantly affect the major and minor complication rates of image-guided percutaneous liver biopsies. In this sense, most guidelines recommend total paracentesis before the biopsy, rather than prohibiting percutaneous liver biopsies in patients with tense ascites. We think that the reason for the low complication rate was mainly due to the use of ultrasonography.

There were several limitations associated with this study mainly due to its retrospective format. Minor complications, such as pain,
could not be fully investigated because of the retrospective design. Therefore, the incidence of complications may be underestimated. Second, since this study was conducted in a single institution, it may not represent all patient types. However, some generalizations are possible because several doctors had performed liver biopsies independently even in a single institution and we examined all patients who underwent liver biopsies in the institution over 18 years, achieving a large study population. To represent the dynamic changes in liver biopsy indications in South Korea, further multicenter studies to validate our results are necessary.

In conclusion, a liver biopsy is an irreplaceable diagnostic method, even in this era of noninvasive techniques. Also, we found that ultrasonography-guided liver biopsy was safe for patients with liver cirrhosis.

Authors’ contribution
Conceptualization: JJ Yoo; methodology: Y Chang and JI Kim; formal analysis: B Lee; investigation: JI Kim; resources: SW Jeong and JJ Jang; data curation: SG Kim; writing — original draft preparation, Y Chang and JJ Yoo; writing — review & editing, JJ Yoo; visualization, B Lee; and supervision, SG Kim and YS Kim.

Acknowledgements
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Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
15. 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.

Effectiveness of nivolumab versus regorafenib in hepatocellular carcinoma patients who failed sorafenib treatment


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Graphical Abstract

Kaplan-Meier curves of overall survival according to the treatment group: Nivolumab may be associated with improved overall survival compared to regorafenib in HCC patients after sorafenib failure.

Adjusted HR 0.34 (95% CI, 0.18–0.65)
P = 0.11 by log-rank test

Abbreviations:
AFP, alpha-fetoprotein; aHR, adjusted hazard ratio; CI, confidence interval; HCC, hepatocellular carcinoma; IPTW, inverse probability treatment weighting; IQR, interquartile range; mRECIST, modified Response Evaluation Criteria in Solid Tumors; NE, not estimable; OS, overall survival; RECIST, Response Evaluation Criteria in Solid Tumors; TTP, time to progression

* These authors contributed equally to this work.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is estimated to be the sixth most commonly diagnosed cancer and the fourth major cause of cancer-related mortality worldwide, with 841,000 new cases and 782,000 deaths annually. Despite the efforts to improve public health, the age-standardized incidence rate is increasing in countries with a high socio-demographic index.

Although sorafenib has been shown to improve overall survival (OS) of patients with advanced HCC, the prolongation of survival is modest. Recently, several therapeutic agents have been tested in patients with HCC who have progressed during sorafenib treatment or who were intolerant to sorafenib. In a placebo-controlled phase 3 study (RESORCE trial) in patients who had disease progression after sorafenib treatment, regorafenib, an oral multi-kinase inhibitor, showed improved OS. Median OS was 10.7 months for regorafenib and 7.9 months for placebo. In a non-comparative phase 2 study with nivolumab (CheckMate 040 trial), which is a human anti-programmed cell death-1 monoclonal antibody that disrupts immune checkpoint signaling, a subgroup analysis in patients who had previously treated with sorafenib demonstrated an objective response rate of 14% and disease control rate of 55%. Moreover, the median duration of response was 19.4 months, suggesting that nivolumab might offer durable tumor responses. Based on these promising results, nivolumab is currently available in several countries, including the United States and Korea, for the treatment of advanced HCC after sorafenib failure.

Though regorafenib and nivolumab have been proven effective in pivotal clinical trials, study results in real-world clinical practice are limited. In addition, a direct comparison of the efficacy of regorafenib and nivolumab has not been conducted to date. As sorafenib failed patients raised importance in clinical practice, we also included sorafenib intolerant patients. In this study, we aimed to evaluate and compare the effectiveness of regorafenib versus nivolumab in patients with HCC who had failed sorafenib treatment.
PATIENTS AND METHODS

Study population

In this retrospective cohort study, we included consecutive HCC patients who had experienced disease progression or intolerance during sorafenib treatment and received regorafenib or nivolumab thereafter between July 1, 2015 and October 31, 2018 at Seoul National University Hospital (Seoul, Korea) (Fig. 1). The study conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital. The requirement to obtain informed consent was waived because existing administrative and clinical data were obtained from the electronic databases and analyzed retrospectively.

We included patients who met all of the following inclusion criteria: 1) were at least 18 years old; 2) had received regorafenib or nivolumab treatment after sorafenib failure; and 3) HCC was radiologically or histologically confirmed according to American Association for the Study of Liver Disease criteria, with measurable disease based on the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). Patients were excluded from the study if any of the following exclusion criteria met: 1) received prior immune-targeted therapy; 2) were participating in clinical trials; 3) prior liver transplantation; 4) unclear history of sorafenib treatment; and 5) had a concurrent malignancy other than HCC.

Treatment

The clinician comprehensively assessed the clinical situation of the patient, including side effects during the previous sorafenib treatment and residual liver function to select the subsequent treatment after sorafenib failure. The initial dose of regorafenib was 160 mg once daily for the first 21 days of each 28-day cycle. Regorafenib dose adjustment by the amount of 40 mg or transient interruption (<14 days) owing to drug-related toxicity was allowed. Nivolumab was administered intravenously every 2 weeks at a dose of 3 mg/kg. The treatments were continued until disease progression, intolerable toxicity, patient refusal of further treatment, or death from any cause.

Evaluation

Baseline demographics and clinical characteristics were summarized by treatment group. It has been reported that the prognosis of patients with HCC depends on both residual liver function and the extent of the tumor. Child-Pugh classification and standard liver function tests were used to evaluate remaining liver function. Clinically significant portal hypertension, defined by a hepatic venous pressure gradient ≥10 mmHg, was estimated by evidence of overt clinical decompensation (i.e., ascites, varices, or hepatic encephalopathy) or presence of splenomegaly with thrombocytopenia (platelet count <150,000/mm$^3$).

Pretreatment intrahepatic
Table 1. Baseline characteristics by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=150)</th>
<th>Regorafenib (n=102)</th>
<th>Nivolumab (n=48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (55–70)</td>
<td>62 (56–71)</td>
<td>61 (54–67)</td>
<td>0.14</td>
</tr>
<tr>
<td>Male</td>
<td>122 (81.3)</td>
<td>83 (81.4)</td>
<td>39 (81.2)</td>
<td>0.99</td>
</tr>
<tr>
<td>HCC etiology</td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>HBV</td>
<td>124 (82.7)</td>
<td>86 (84.3)</td>
<td>38 (79.2)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>26 (17.3)</td>
<td>16 (15.7)</td>
<td>10 (20.8)</td>
<td></td>
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<tr>
<td>Child-Pugh score</td>
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<td></td>
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<td>0.003</td>
</tr>
<tr>
<td>5</td>
<td>86 (57.3)</td>
<td>66 (64.7)</td>
<td>20 (41.7)</td>
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</tr>
<tr>
<td>6</td>
<td>51 (34.0)</td>
<td>32 (31.4)</td>
<td>19 (39.6)</td>
<td></td>
</tr>
<tr>
<td>7–9</td>
<td>13 (8.7)</td>
<td>4 (3.9)</td>
<td>9 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>66 (44.0)</td>
<td>42 (41.2)</td>
<td>24 (50.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Biliary invasion</td>
<td>7 (4.7)</td>
<td>5 (4.9)</td>
<td>2 (4.2)</td>
<td>0.84</td>
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<tr>
<td>Extrahepatic tumor burden</td>
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<td>0.40</td>
</tr>
<tr>
<td>None</td>
<td>25 (16.7)</td>
<td>19 (18.6)</td>
<td>6 (12.5)</td>
<td></td>
</tr>
<tr>
<td>&lt;50%</td>
<td>93 (62.0)</td>
<td>64 (62.7)</td>
<td>29 (60.4)</td>
<td></td>
</tr>
<tr>
<td>≥50%</td>
<td>32 (21.3)</td>
<td>19 (18.6)</td>
<td>13 (27.1)</td>
<td></td>
</tr>
<tr>
<td>Extrahepatic metastases</td>
<td>120 (80.0)</td>
<td>79 (77.5)</td>
<td>41 (85.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>Lymph node</td>
<td>54 (36.0)</td>
<td>37 (36.3)</td>
<td>17 (35.4)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>77 (51.3)</td>
<td>46 (45.1)</td>
<td>31 (64.6)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>32 (21.3)</td>
<td>21 (20.6)</td>
<td>11 (22.9)</td>
<td></td>
</tr>
<tr>
<td>Peritoneum</td>
<td>24 (16.0)</td>
<td>16 (15.7)</td>
<td>8 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3 (2.0)</td>
<td>1 (1.0)</td>
<td>2 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Portal hypertension</td>
<td>64 (42.7)</td>
<td>42 (41.2)</td>
<td>22 (45.8)</td>
<td>0.59</td>
</tr>
<tr>
<td>BCLC stage</td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>B</td>
<td>5 (3.3)</td>
<td>4 (3.9)</td>
<td>1 (2.1)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>145 (96.7)</td>
<td>98 (96.1)</td>
<td>47 (97.9)</td>
<td></td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7 (3.3–4.0)</td>
<td>3.8 (3.4–4.1)</td>
<td>3.7 (3.2–3.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.0 (0.7–1.4)</td>
<td>1.0 (0.7–1.3)</td>
<td>1.1 (0.7–1.7)</td>
<td>0.29</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>49 (33–80)</td>
<td>45 (32–77)</td>
<td>60 (39–90)</td>
<td>0.16</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>37 (22–59)</td>
<td>37 (22–57)</td>
<td>38 (23–60)</td>
<td>0.42</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>138 (88–220)</td>
<td>133 (87–220)</td>
<td>144 (92–212)</td>
<td>0.88</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.81 (0.68–0.93)</td>
<td>0.82 (0.68–0.93)</td>
<td>0.80 (0.69–0.93)</td>
<td>0.44</td>
</tr>
<tr>
<td>Platelet count (×10^{3}/L)</td>
<td>154 (105–218)</td>
<td>164 (107–228)</td>
<td>149 (101–204)</td>
<td>0.28</td>
</tr>
<tr>
<td>INR</td>
<td>1.08 (1.02–1.15)</td>
<td>1.06 (1.00–1.12)</td>
<td>1.10 (1.04–1.21)</td>
<td>0.002</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>431 (12.5–4,185.0)</td>
<td>338.0 (11.9–3,799.3)</td>
<td>760.0 (18.4–4,665.0)</td>
<td>0.90</td>
</tr>
<tr>
<td>PIVKA (mAU/mL)</td>
<td>1,453 (135–8,898)</td>
<td>1,365 (149–8,699)</td>
<td>1,803 (107–10,545)</td>
<td>0.48</td>
</tr>
<tr>
<td>MoRAL</td>
<td>570.6 (205.1–1,254.3)</td>
<td>570.6 (240.1–1,224.0)</td>
<td>559.5 (234.2–1,276.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Intolerant to sorafenib</td>
<td>13 (8.7)</td>
<td>6 (5.9)</td>
<td>7 (14.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>Duration of sorafenib* (months)</td>
<td>2.8 (2.0–4.7)</td>
<td>3.0 (2.3–6.2)</td>
<td>2.5 (1.4–3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time interval between sorafenib and treatment (months)</td>
<td>1.2 (0.0–4.7)</td>
<td>0.9 (0.0–4.6)</td>
<td>1.8 (0.3–5.8)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

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

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; INR, international normalized ratio; AFP, alpha-fetoprotein; PIVKA, protein induced by vitamin K absence or antagonist; MoRAL, model to predict tumor recurrence after living donor liver transplantation.

*Assessed for patients who progressed after sorafenib (n=136).
tumor burden and extrahepatic spread were assessed based on an imaging study prior to treatment initiation. A model to predict tumor recurrence after living donor liver transplantation score (\(=11 \times \text{protein induced by vitamin K absence-II} + 2 \times \alpha \text{-fetoprotein (AFP)}\)), which was developed and validated previously to have prognostic value in patients undergoing liver transplantation for HCC was calculated for the individual patient and included in the further analyses.\(^{20,21}\)

**Clinical outcome measures**

The date of the first regorafenib or nivolumab administration was assigned as the index date. The primary endpoint was OS, defined as time from the index date to death from any cause. For patients who switched to the other treatment (i.e., regorafenib after nivolumab or nivolumab after regorafenib), the follow-up was censored at the date of initiation of the subsequent treatment. Secondary endpoints were time to progression (TTP) defined as time from the index date to radiologic progression, objective response rate, duration of response, disease control rate. To evaluate radiologic response, two independent radiologists reviewed the images (abdomen computed tomography or magnetic resonance imaging), and the clinicians read the same images independently based on modified RECIST (mRECIST).\(^{22}\) Every patient underwent scheduled imaging follow-up every 2–3 months. Considering insufficient drug exposure, imaging studies within 4 weeks of treatment initiation were precluded for the assessment of tumor response. Subgroup analyses were conducted for the primary and secondary endpoints according to baseline characteristics. Treatment-related toxicities leading to premature discontinuation of the treatment before the documentation of disease progression were investigated.

**Statistical analyses**

Baseline demographic and clinical characteristics were compared between treatment groups. For group-wise comparisons, the independent samples \(t\)-test was used for continuous variables and either the chi-square test or the Fischer’s exact test was applied for categorical variables.

The Kaplan-Meier method was used for analyses of OS and TTP. Differences between treatment groups were verified by log-rank test. To investigate whether results were confounded by other risk factors for OS or TTP, we used the multivariable Cox regression analysis. Variables in the multivariable analyses were selected using stepwise regression with the forward selection method. The objective response rate and disease control rate in each group were compared using the Cochran-Mantel-Haenszel test. Inverse probability treatment weighting (IPTW) based on the propensity score was applied to mitigate baseline differences between the groups.\(^{21,24}\) The propensity score for each patient was calculated using a logistic regression model using baseline characteristics, such as age, Child-Pugh score, and tumor stage. Moreover, we employed weighted Cox proportional hazards regression model to identify the treatment effect on OS and TTP.

All statistical analyses were performed using R software (version 3.6.2).
RESULTS

Patient population

Among 150 HCC patients included in final analyses, 102 patients were treated with regorafenib and 48 received nivolumab. The baseline demographic and clinical characteristics of the study patients by the group are shown in Table 1. The median age was 62 (interquartile range [IQR], 55–70) years overall, and was not significantly different between the groups (P=0.14). Male patients accounted for 81.3% of patients, and most study patients (82.7%) were chronically infected with hepatitis B virus. Almost all patients (96.7%) were Barcelona Clinic Liver Cancer stage C, and there was no statistical difference between the groups. While 25 patients (16.7%) had no intrahepatic tumor, >50% of the liver was occupied with the tumor in 32 patients (21.3%) at baseline.

### Table 2. Univariable and multivariable Cox regression analyses for overall survival

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Nivolumab (vs. regorafenib)</td>
<td>1.081 (0.644–1.813)</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>0.987 (0.966–1.009)</td>
<td>0.24</td>
</tr>
<tr>
<td>Male sex (vs. female)</td>
<td>2.313 (1.052–5.086)</td>
<td>0.04</td>
</tr>
<tr>
<td>Etiology of HCC, HBV (vs. others)</td>
<td>1.572 (0.775–3.185)</td>
<td>0.21</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.532 (2.009–6.210)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7–9</td>
<td>6.264 (2.926–13.410)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular invasion, yes (vs. no)</td>
<td>2.104 (1.275–3.471)</td>
<td>0.004</td>
</tr>
<tr>
<td>Biliary invasion, yes (vs. no)</td>
<td>3.049 (1.205–7.718)</td>
<td>0.02</td>
</tr>
<tr>
<td>Intrahepatic tumor burden</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>None</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>&lt;50%</td>
<td>2.024 (0.847–4.833)</td>
<td>0.11</td>
</tr>
<tr>
<td>≥50%</td>
<td>7.148 (2.853–17.905)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Extrahepatic metastasis, yes (vs. no)</td>
<td>0.522 (0.296–0.919)</td>
<td>0.02</td>
</tr>
<tr>
<td>Portal hypertension, yes (vs. no)</td>
<td>1.503 (0.919–2.458)</td>
<td>0.10</td>
</tr>
<tr>
<td>BCLC stage C (vs. B)</td>
<td>0.913 (0.222–3.759)</td>
<td>0.90</td>
</tr>
<tr>
<td>AST (per IU/L)</td>
<td>1.012 (1.008–1.016)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (per IU/L)</td>
<td>1.008 (1.004–1.012)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALP (per IU/L)</td>
<td>1.003 (1.002–1.004)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (per mg/dL)</td>
<td>0.359 (0.113–1.202)</td>
<td>0.08</td>
</tr>
<tr>
<td>Platelet count (per ×10^3/L)</td>
<td>1.002 (1.000–1.005)</td>
<td>0.09</td>
</tr>
<tr>
<td>AFP ≥400 ng/mL</td>
<td>1.359 (0.828–2.232)</td>
<td>0.23</td>
</tr>
<tr>
<td>PIVKA ≥1,000 mAU/mL</td>
<td>2.842 (1.622–4.980)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MoRAl ≥314.8</td>
<td>3.056 (1.677–5.569)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P values were determined using Cox proportional hazards regression models. P<0.05 indicated a significant difference.

HR, hazards ratio; CI, confidence interval; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AFP, alpha-fetoprotein; PIVKA, protein induced by vitamin K absence or antagonist; MoRAl, model to predict tumor recurrence after living donor liver transplantation.
Most patients (91.3%) were Child-Pugh class A, and the proportion of patients with Child-Pugh class B was significantly higher in the nivolumab group ($P=0.003$). Prothrombin time in international normalized ratio was significantly higher in the nivolumab group ($P=0.002$). Mean serum albumin levels were lower in the nivolumab group, but the difference was not statistically significant ($P=0.08$). Thirteen (8.7%) patients who were intolerant to sorafenib treatment were included in this study. The proportion of sorafenib-intolerant patients was higher in the nivolumab group than in the regorafenib group (14.6% vs. 5.9%).

During the follow-up period, all of the patients in the nivolumab group received prespecified fixed dose. There was only one patient (2%) who experienced treatment interruption. Sixty-five patients (64%) required dose modification or treatment interruption due to adverse events in the regorafenib group. Excluding treatment delays or interruptions, 60 patients (59%) received full dose without dose reduction, and 22 patients (22%) received reduced dose (<120 mg) of regorafenib.

### OS

At the cutoff date for the final analysis (January 15, 2019), the median duration of follow-up of the entire cohort was 5.0 months (IQR, 2.9–7.5): 4.3 months (IQR, 2.9–6.6) for the regorafenib group and 5.2 months (IQR, 3.1–9.7) for the nivolumab group. Overall, 65 patients (43.3%) died during the follow-up period: 38 (37.3%) of 102 patients in the regorafenib group and 27 (56.3%) of 48 patients in the nivolumab group. Median OS was 5.9 months (95% confidence interval [CI], 3.2–18.1) in the nivolumab group and 6.9 months (95% CI, 3.5–13.1) months in the regorafenib group ($P=0.77$ by log-rank test) (Fig. 2A). In the multivariable analysis, treatment with nivolumab was independently associated with improved OS compared to regorafenib after adjusting for confounding factors, including Child-Pugh score and intrahepatic tumor burden (adjusted hazard ratio [aHR], 0.54; 95% CI, 0.30–0.96; $P=0.04$) (Table 2). After balancing the baseline characteristics by IPTW, there was no longer any significant difference between the treatment groups (Supplementary Table 1). Although there was no statistically significant difference between the groups, nivolumab was associated with better OS than regorafenib ($P=0.11$ by log-rank test) (Fig. 3A). Weighted Cox proportional hazards model also revealed that the nivolumab treatment was associated with improved OS vs. regorafenib; aHR, 0.34; 95% CI, 0.18–0.65; $P=0.001$) (Supplementary Table 2). Similar results were observed in additional analysis without censoring the patient who switched to the other treatment (Supplementary Fig. 1).

### TTP

During the follow-up period, tumors progressed radiologically in 91 (60.7%) of 150 patients based on mRECIST: 62 (60.8%) of 102 patients in the regorafenib group and 29 (60.4%) of 48 patients.
in the nivolumab group. The median TTP was not significantly different between the nivolumab group (4.0 months; 95% CI, 1.8–8.7) and the regorafenib group (3.3 months; 95% CI, 2.0–5.3) \((P=0.40\) by log-rank test) (Fig. 2B). In the multivariable analysis, there was no significant association with improved TTP between treatments (nivolumab vs. regorafenib; aHR, 0.81; 95% CI, 0.51–1.30; \(P=0.48\)) (Table 3). After IPTW, there was no significant difference in TTP between the groups \((P=0.30\) by log-rank test) (Fig. 3B), and treatment group was not an independent predictor for TTP (nivolumab vs. regorafenib; aHR, 0.74; 95% CI, 0.39–1.41; \(P=0.36\)) (Supplementary Table 3).

### Overall tumor response

No patient in either treatment group achieved a complete response. Eight (16.7%) of 48 patients in the nivolumab group and six (5.9%) of 102 patients in the regorafenib group achieved a partial response by mRECIST, and nivolumab showed significantly better objective response rate compared to regorafenib \((P=0.041)\) (Table 4). The disease control rate (i.e., the proportion of patients who had an objective response or disease stabilization) was 50.0% in the nivolumab group and 47.1% in the regorafenib group \((P=0.58)\). Among the 14 patients who achieved an objec-

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**Table 3.** Univariable and multivariable Cox regression analyses for time to progression

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>(P)-value</td>
</tr>
<tr>
<td>Nivolumab (vs. regorafenib)</td>
<td>0.824 (0.522–1.299)</td>
<td>0.40</td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>0.985 (0.964–1.006)</td>
<td>0.15</td>
</tr>
<tr>
<td>Male sex (vs. female)</td>
<td>1.375 (0.809–2.335)</td>
<td>0.24</td>
</tr>
<tr>
<td>Etiology of HCC, HBV (vs. others)</td>
<td>1.458 (0.792–2.681)</td>
<td>0.23</td>
</tr>
<tr>
<td>Child-Pugh score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.502 (0.954–2.366)</td>
<td>0.08</td>
</tr>
<tr>
<td>7–9</td>
<td>0.972 (0.351–2.689)</td>
<td>0.96</td>
</tr>
<tr>
<td>Vascular invasion, yes (vs. no)</td>
<td>1.016 (0.663–1.558)</td>
<td>0.94</td>
</tr>
<tr>
<td>Biliary invasion, yes (vs. no)</td>
<td>1.666 (0.672–4.131)</td>
<td>0.27</td>
</tr>
<tr>
<td>Intrahepatic tumor burden</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>&lt;50%</td>
<td>1.098 (0.642–1.878)</td>
<td>0.73</td>
</tr>
<tr>
<td>≥50%</td>
<td>2.067 (1.073–3.982)</td>
<td>0.03</td>
</tr>
<tr>
<td>Extrahepatic metastasis, yes (vs. no)</td>
<td>0.810 (0.475–1.379)</td>
<td>0.44</td>
</tr>
<tr>
<td>Portal hypertension, yes (vs. no)</td>
<td>1.332 (0.869–2.041)</td>
<td>0.19</td>
</tr>
<tr>
<td>BCLC stage C (vs. B)</td>
<td>0.305 (0.109–0.856)</td>
<td>0.02</td>
</tr>
<tr>
<td>AST (per IU/L)</td>
<td>1.006 (1.001–1.011)</td>
<td>0.01</td>
</tr>
<tr>
<td>ALT (per IU/L)</td>
<td>1.003 (0.998–1.009)</td>
<td>0.23</td>
</tr>
<tr>
<td>ALP (per IU/L)</td>
<td>1.002 (1.001–1.003)</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (per mg/dL)</td>
<td>1.002 (1.001–1.003)</td>
<td>0.002</td>
</tr>
<tr>
<td>Platelet count (per ×10^9/L)</td>
<td>0.589 (0.230–1.510)</td>
<td>0.27</td>
</tr>
<tr>
<td>AFP ≥400 ng/mL</td>
<td>1.063 (0.703–1.608)</td>
<td>0.77</td>
</tr>
<tr>
<td>PIVKA ≥1,000 mAU/mL</td>
<td>1.217 (0.803–1.846)</td>
<td>0.36</td>
</tr>
<tr>
<td>MoRAL ≥314.8</td>
<td>1.210 (0.794–1.844)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

\(P\) values were determined using Cox proportional hazards regression models. \(P<0.05\) indicated a significant difference.

HR, hazards ratio; CI, confidence interval; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AFP, alpha-fetoprotein; PIVKA, protein induced by vitamin K absence or antagonist; MoRAL, model to predict tumor recurrence after living donor liver transplantation.

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Nivolumab versus regorafenib for advanced HCC
Subgroup analysis

Comparisons of outcomes between treatments were assessed among the subgroups. There was no difference in OS or TTP between the groups regarding duration of prior sorafenib treatment (Supplementary Fig. 2), or Child Pugh score (Supplementary Fig. 3). Further analysis according to the presence or absence of portal hypertension/macrovascular invasion, and AFP level showed no significant OS difference between the two treatment groups (Supplementary Fig. 4).

Safety

The reasons for the drug discontinuation were assessed for the safety analysis. Eighty-three patients discontinued the treatment due to disease progression among 122 patients who discontinued the treatment (Supplementary Table 4). Adverse events that caused premature drug discontinuation occurred in 24 patients (23.5%) in the regorafenib group and eight patients (16.7%) in the nivolumab group (P=0.34). The major cause of drug discontinuation before radiologic progression was hepatic decompensation: four (8.3%) in the nivolumab group and 10 (9.8%) in the regorafenib group (P=0.77). Subsequent treatments after study drug failure were assessed. The proportion of patients who received any treatments after the study drug failure in both groups was similar (P=0.54) (Supplementary Table 5).

DISCUSSION

In the current study in HCC patients who had experienced sorafenib treatment failure, nivolumab treatment was associated with improved OS compared with regorafenib treatment. The risk of all-cause mortality was reduced by 46% in patients receiving nivolumab compared to patients receiving regorafenib. Although TTP did not differ significantly between the treatments, nivolumab demonstrated a significantly better objective response rate than regorafenib. Both drugs were well tolerated during the treatment period; however, adverse events leading to drug discontinuation before radiologic disease progression were more frequent in patients receiving regorafenib.

To date, this is the first study in a real-world setting comparing the effectiveness of regorafenib and nivolumab treatments in HCC patients who previously failed sorafenib treatment. Nivolumab was more effective than regorafenib in terms of OS. While median OS was longer in patients treated with regorafenib (6.9 months) versus nivolumab (5.9 months), nivolumab showed a significant association with improved OS after adjusting for confounders. In the present study, patients with higher Child-Pugh scores at baseline, an independent predictive factor for poor OS, were more likely to be treated with nivolumab. To minimize treatment selection bias, we adopted IPTW in addition to multivariable analyses, and the association of nivolumab with prolonged OS was confirmed.

The higher objective response rate with nivolumab might confer its association with improved OS compared with regorafenib. The objective response rate with nivolumab was 16.7% in the current study, a finding that is consistent with previous studies reporting objective response rates of 14.3–16.7%.5,10,14,25 However, the objective response rate with regorafenib (5.9%) was lower compared to the previous studies (10.0–10.6%).5,13 In the RESORCE trial, the objective response rate was 10.6%, and 68% of patients experienced drug interruption or dose modification during the treatment.5 The rates of treatment interruption or dose modification of patients receiving regorafenib in our present study was similar to those in the RESORCE trial. Dose intensity was also comparable. Scheduled imaging was performed every 6 weeks at...
the beginning of treatment in the RESORCE trial. However, the imaging intervals were variable ranging from 2 to 3 months in the current retrospective study, which may have affected the tumor response. The duration of response was longer in patients treated with nivolumab than in patients treated with regorafenib, even though the difference was not statistically significant. This finding is consistent with the results of the CheckMate 040 trial which reported sustained tumor response with nivolumab in HCC patients. Further studies with sufficient observational periods are warranted to demonstrate a statistically significant difference in the duration of response between nivolumab and regorafenib.

The median OS with nivolumab (5.9 months) and regorafenib (6.9 months) was shorter than reported in the corresponding results from the pivotal trials. Median OS with regorafenib was 10.7 months in the RESORCE trial, and median OS with nivolumab was 15.1 months in the sorafenib progressor cohort in the CheckMate 040 trial. In contrast, recent real-world evidence studies evaluating the efficacy of nivolumab reported that median OS with nivolumab ranged from <2 months to 11.0 months. Considering the major causes of death of advanced HCC patients were intrahepatic tumor progression and complications of cirrhosis, the discrepancy between the results of clinical trials and real-world studies can be explained by the differences in baseline characteristics of the study population. However, even though regorafenib treatment demonstrated efficacy over placebo in prolonging OS after sorafenib failure in the RESORCE trial, and the ability of nivolumab to improve OS compared to regorafenib was demonstrated in the current study, survival gain with nivolumab treatment is still unsatisfactory. Recently, the efficacy of combination treatments with immune checkpoint inhibitors plus molecular targeted agents in advanced HCC patients was evaluated in phase 1b trials. Promising results of objective response rates over 30–40% were reported; however, safety-related issues need to be clarified. In a recent study, it was reported that less than half of patients were eligible for subsequent systemic therapy after sorafenib failure due to impaired hepatic function. Therefore, it is crucial to establish a treatment strategy maximizing efficacy without compromising safety.

Despite the unsatisfactory effects, it is noteworthy that small portion of patients did achieve prolonged DOR more than a year and even complete responses with regorafenib or nivolumab treatment. HCC patients who experienced sorafenib failure are heterogeneous in terms of varying prior treatments, different tumor characteristics, and degrees of liver fibrosis, but biomarker based treatment is limited due to the risk of liver biopsy. Thus, treatment strategy of those patients is mainly dependent on clinical judgment. Previous study by Lee et al. estimated survival and prognosis of patients who experienced sorafenib failure based on clinical parameters. In our study, male sex, extensive intrahepatic tumor burden and higher Child-Pugh score at the time of sorafenib failure were identified as poor survival markers.

The safety profiles of regorafenib and nivolumab in the present study were generally consistent with those reported in previous studies. Whilst both treatments showed comparable safety in terms of drug discontinuation rate, the proportion of patients with impaired hepatic function was significantly higher among patients receiving nivolumab compared to regorafenib at baseline. Furthermore, three of four patients who discontinued nivolumab treatment due to hepatic decompensation had extensive intrahepatic tumor burden (>50%) at the start of treatment. Therefore, the possibility of overestimating the hepatotoxicity of nivolumab should be accounted for. Previous studies including Child-Pugh class B patients with HCC showed similar safety profile of nivolumab between Child-Pugh class A and Child-Pugh Class B subgroups. Moreover, one patient with Child-Pugh class C disease at baseline showed complete response during the treatment with nivolumab in a small study involving 14 HCC patients. The safety of nivolumab in patients with hepatic impairment needs to be validated in future studies.

Our study has several limitations to be considered. First, in terms of tumor response assessment, the follow-up intervals for imaging studies varied among the study patients. However, since this issue results from the nature of the retrospective study design and was not limited to either regorafenib or nivolumab treatment, the effect on the efficacy comparison is thought to be negligible. Second, although the major adverse events leading to treatment discontinuation were analyzed, it was impossible to obtain accurate information about adverse events that occurred during the study period. Further, well-designed prospective studies are needed to verify the safety in real-world clinical practice. Third, because we assessed the tumor response based on mRECIST, pseudoprogression, a unique pattern of tumor response with immunotherapy, was not considered for the patients receiving nivolumab. Though the rate of pseudoprogression is known to be rare (<10%) across tumor types, there have been reports of prolonged response to immune checkpoint inhibitors after pseudoprogression in HCC patients. In the era of cancer immunotherapy, tumor response evaluation based on immune RECIST should be further validated in HCC patients.

In the current study in patients with HCC who previously failed
sorafenib treatment, subsequent nivolumab treatment may improve OS over regorafenib treatment and also demonstrated a higher objective response rate. Since nivolumab, an immune modulator, has a completely different mechanism of action and side effect profile from sorafenib, treatment with nivolumab may be a potential therapeutic option for the treatment of HCC after sorafenib failure, particularly in patients showing sorafenib intolerance. Both regorafenib and nivolumab were well tolerated overall. Additional studies investigating the effectiveness of nivolumab in combination with other treatments are warranted in consideration of its efficacy and safety.

**Author’s contribution**

The corresponding author (Yoon Jun Kim) had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

1) Study concept and design: Cheol-Hyung Lee, Yun Bin Lee, and Yoon Jun Kim
3) Collection and assembly of data: Cheol-Hyung Lee, Minseok Albert Kim, Heejoon Jang, Hyunwoo Oh, Sun Woong Kim
4) Data analysis and interpretation: Cheol-Hyung Lee, Yun Bin Lee, and Yoon Jun Kim
5) Manuscript writing: Cheol-Hyung Lee, Yun Bin Lee, and Yoon Jun Kim
6) Final approval of manuscript: All authors

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**Conflicts of Interest**

Dr. Lee YB reports receiving research grant from Samjin Pharmaceuticals; Dr. Lee KH reports honoraria from Roche and AstraZeneca, and serves an advisory role for AstraZeneca, Samsung Bioepis, BAYER, Roche, Eisai, and Ono Pharmaceutical; Dr. Lee JH reports receiving lecture fee from GreenCross Cell, Daewoong Pharmaceuticals, and Gilead Korea; Dr. Yu SJ reports lecture fee from Bayer HealthCare Pharmaceuticals; Dr. Kim YJ, research grants from Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals, Samjin Pharmaceuticals, AstraZeneca, and Pharmaking, and lecture fees from Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, and Handok Pharmaceuticals; Dr. Yoon JH, research grants from AstraZeneca, Bayer HealthCare Pharmaceuticals, Daewoong Pharmaceuticals, and Bukwang Pharmaceuticals. No other potential conflict of interest relevant to this article was reported.

**SUPPLEMENTARY MATERIAL**

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

**REFERENCES**


http://www.e-cmh.org https://doi.org/10.3350/cmh.2019.0049n
Comparison of LI-RADS 2018 and KLCA-NCC 2018 for noninvasive diagnosis of hepatocellular carcinoma using magnetic resonance imaging

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Graphical Abstract

ECA-MRI

LI-RADS 2018 -LR-5
KLCA-NCC 2018 -definite HCC

AP
PVP or DP
PVP or DP

Sensitivity: LI-RADS \approx KHCA-NCC 2018
Specificity: LI-RADS \approx KLCA-NCC 2018

HBA-MRI

LI-RADS 2018 -LR-5
KLCA-NCC 2018 -definite HCC

AP
PVP or DP
PVP or DP

Sensitivity: LI-RADS < KLCA-NCC 2018
Specificity: LI-RADS \approx KLCA-NCC 2018

Abbreviations:
AP, arterial phase; cHCC-CCA, combined hepatocellular carcinoma-cholangiocarcinoma; DP, delayed phase; DWI, diffusion-weighted imaging; ECA, extracellular contrast agent; HBA, hepatobiliary agent; HBP, hepatobiliary phase; HCC, hepatocellular carcinoma; KLCA, Korean Liver Cancer Association; KLCSG, Korean Liver Cancer Study Group; LI-RADS, Liver Imaging Reporting and Data System; MRI, magnetic resonance imaging; NCC, National Cancer Center; PVP, portal venous phase; TP, transitional phase; US, ultrasound

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer.\(^1\) It is the only malignancy of which the clinical practice for diagnosis is to use imaging without pathological confirmation in high-risk patients; thus, reliable noninvasive imaging criteria are crucial.\(^2\)\(^3\) To achieve consistent diagnosis of HCC, several scientific organizations have proposed imaging-based diagnostic criteria. The imaging criteria differ between geographic areas, reflecting differences in clinical environment and treatment strategies.\(^4\)\(^5\)

The Liver Imaging Reporting and Data System (LI-RADS), endorsed by the American College of Radiology, is a comprehensive system to standardize the terminology, technique, interpretation, reporting, and data collection of liver imaging in patients at risk for HCC.\(^6\) LI-RADS is currently the most widely used noninvasive diagnostic criteria for HCC in radiological studies because it provides detailed definitions for major and ancillary imaging features of HCCs. LI-RADS categorizes an observation according to its probability of HCC (ranging from LR-1 to LR-5) or malignancy but not specific for HCC (LR-M), on the basis of major, ancillary, and LR-M imaging features.\(^6\) LI-RADS 2018 represents the fourth update and has been fully integrated into the American Association for the Study of Liver Disease 2018 HCC Practice Guidance.\(^2\) LI-RADS was developed and modified predominantly based on data from Western countries and was designed to emphasize the specificity and positive predictive value over sensitivity for liver transplant eligibility.\(^4\)\(^5\)

The Korean Liver Cancer Study Group (KLCSG)-National Cancer Center (NCC) HCC practice guidelines were first announced in 2003 and have been revised in 2009 and 2014.\(^7\)\(^8\) Since then, the Korean Liver Cancer Association (KLCA, formerly KLCSG)-NCC published KLCA-NCC 2018 practice guidelines for the management of HCC.\(^9\) They provide diagnosis and treatment guideline specific to the Asian who manifest different clinical behaviors of
HCC compared to Western population, especially in Korean. The KLCA-NCC 2018 practice guidelines for the imaging diagnosis of HCC has been revised into a non-binary decision algorithm that covers the whole spectrum of observations, similar to LI-RADS. The KLCA-NCC 2018 guidelines categorize an observation into indeterminate, probable HCC, and definite HCC after excluding benign lesions such as cyst or hemangiomas based on bright T2 signal intensity and other malignancies based on targetoid appearance. KLCA-NCC 2018 was designed to favor high sensitivity for the detection of early HCC and early treatment with locoregional therapies.

However, data to support these updated versions of the imaging criteria are insufficient; moreover, limited data are available to compare the LI-RADS 2018 and KLCA-NCC 2018 criteria for the noninvasive diagnosis of HCC. Therefore, this study aimed to compare the diagnostic performances of LI-RADS 2018 and KLCA-NCC 2018 criteria on magnetic resonance imaging (MRI) for diagnosing HCC in high-risk patients.

**MATERIALS AND METHODS**

This retrospective study was approved by the Institutional Review Board of Severance Hospital (IRB number 4-2019-1127) and the requirement for informed consent was waived.

**Patients**

We searched our institution’s databases for a clinical cohort under HCC surveillance and identified 2,576 patients who underwent MRI for further diagnostic workup between January 2016 and December 2017. The inclusion criteria were as follows: 1) age of 18 years or older; 2) patients at high risk for HCC with cirrhosis or chronic hepatitis B; 3) presence of at least one and up to five hepatic lesions (each ≥1 cm) on MRI; and 4) no previous treatment for hepatic lesions. The exclusion criteria were as follows: 1) patients with cirrhosis due to congenital hepatic fibrosis or sporadic, hypertrophic pseudomass, confluent fibrosis, and focal scar. However, we include hemangiomas in our study. A radiologist (S.K., with 9 years of experience in abdominal radiology) who was not involved in the image analysis for this study finalized the study population and selected hepatic lesions.

**MRI examination**

MRI was performed using 3.0-T systems (Magnetom Trio Tim, Siemens Healthineers, Erlangen, Germany; Intera Achieva, Inge- ria, or Ingenia CX, Philips Healthcare, Best, the Netherlands; and Discovery MR 750w, GE Healthcare, Waukesha, WI, USA). The protocol included acquisition of dual-echo T1-weighted gradient-echo images (in-phase and opposed-phase), T1-weighted 3-dimensional gradient-echo images with dynamic contrast enhancement, navigator-triggered single- or multi-shot T2-weighted images, and diffusion-weighted images at b-values of 0 or 50, 400, and 800 s/mm². Dynamic T1-weighted imaging was performed before and after administering one of the two extracellular contrast agents (ECAs) (gadoterate meglumine, Dotarem, Guerbet SA, Aulnay-sous-Bois, France; or gadobutrol, Gadovist, Bayer Pharma AG, Berlin, Germany) or a hepatobiliary agent (HBA) (gadoxetate disodium, Primovist, Bayer Pharma AG): a bolus injection of 0.2 mL/kg of gadoterate meglumine (n=67) at a rate of 2.0 mL/s or 0.1 mL/kg of gadobutrol (n=4) at a rate of 1.0 mL/s, or 0.1 mL/kg gadoxetate disodium (n=202) at a rate of 1.0 mL/s, followed by a 20-mL saline flush using a power injector. The choice of MRI contrast agents (ECA or HBA) was made at the discretion of the physicians. The physicians were fully informed by radiologists regarding the advantages and disadvantages of each contrast material at a multidisciplinary conference in our institution, such as the potential differences in terms of degree of arterial phase (AP) enhancement and relative frequency of artifacts between the two contrast materials, potential benefits of hepatobiliary phase (HBP) images, and cost of contrast materials. AP scanning was initiated using the test-bolus or bolus-tracking technique. Thereafter, portal venous phase (PVP) and delayed phase (DP) images were obtained by ECA-MRI, while PVP, transitional phase (TP), and 20-minute HBP images were obtained by HBA-MRI. The detailed parameters of the MRI sequences are presented in Supplementary Table 1.

**Lesion registry**

A radiologist (S.K., with 9 years of experience in abdominal ra-
biology) who was not involved in the image analysis bookmarked individual lesions, reported the lesion size and location, and provided a list for further review. The prior computed tomography or MRI examination was used to assess threshold or subthreshold growth, and ultrasound (US) was used to assess US visibility as a discrete nodule when available.

**Image analysis**

Two board-certified abdominal radiologists (M.J.K. and S.L. with 27 and 8 years of experience in liver imaging, respectively) who did not participate in the patient and lesion selection reviewed all images. They were blinded to the final diagnosis of each lesion but were informed that the study population consisted of patients at high risk for HCC. Both LI-RADS 2018 and KLCA-NCC 2018 criteria were independently applied to each lesion. After independent categorization, the inter-reader agreement was evaluated. Discrepancies between the readers were resolved by consensus. Consensus data were used for the main image analysis results.

**LI-RADS 2018**

According to LI-RADS 2018, the readers reviewed the presence or absence of major, ancillary, and LR-M imaging features (Supplementary Table 2). Each lesion was assigned to one of the following categories: LR-1 for definitely benign, LR-2 for probably benign, LR-3 for intermediate probability of malignancy, LR-4 for probably HCC, LR-5 for definitely HCC, LR-M for probably or definitely malignant but not specific for HCC, and LR-TIV for definite tumor in vein. The LI-RADS 2018 category could be adjusted by applying ancillary features and then tie-breaking rules, if needed.

**KLCA-NCC 2018**

According to KLCA-NCC 2018, the readers assessed the presence or absence of major and ancillary imaging features and targetoid appearance (Supplementary Table 3). Each lesion was categorized as definite HCC, probable HCC, indeterminate, benign, and targetoid appearance. Definite HCC was defined as AP hyperenhancement with washout in the PVP, DP, or HBP. In a lesion with some but not all of the major imaging features of HCC, probable HCC was assigned only when the lesion fulfilled at least one item from each of the following two categories of ancillary imaging features: favoring malignancy in general, and favoring HCC in particular. The diagnostic criteria for HCC should be applied in a stepwise manner after excluding marked T2 hyperintensity or targetoid appearance. When the imaging diagnosis was inconclusive, a lesion was defined as indeterminate. Although the KLCA-NCC 2018 guidelines do not clearly define benign and other malignancy categories, we added benign lesions and targetoid appearance to the system in order to match the classification with the LI-RADS 2018. A lesion that fulfilled at least one of the ancillary features favoring benignity according to KLCA-NCC 2018 was classified as a benign lesion. Targetoid appearance was evaluated on diffusion-weighted imaging (DWI) or contrast-enhanced sequences.

**Comparison of LI-RADS 2018 and KLCA-NCC 2018**

Definite HCC of KLCA-NCC 2018 corresponds to LR-5 of LI-RADS 2018, and probable HCC of KLCA-NCC 2018 corresponds to LR-4 of LI-RADS 2018. For the noninvasive diagnosis of HCC, definite HCC of KLCA-NCC 2018 was compared with LR-5 of LI-RADS 2018, and definite or probable HCC (definite/probable HCC) of KLCA-NCC 2018 was compared with LR-5 or LR-4 (LR-5/4) of LI-RADS 2018.

**Reference standards**

The diagnoses of HCCs and non-HCC malignancies were based on pathology including surgical resection (n=285) or explant for transplantation (n=15). Benign diagnoses were obtained by pathology (n=14) or typical imaging features and stability at imaging for at least 2 years (n=38). The mean interval between the MRI and pathological diagnosis was 20.2 days (range, 0–90 days).

**Statistical analysis**

The baseline characteristics of patients and lesions were compared between the ECA-MRI and HBA-MRI groups using the Fisher’s exact test for categorical variables and the two-sample t test or Mann-Whitney U test for continuous variables. The per-lesion diagnostic performances of LI-RADS 2018 and KLCA-NCC 2018 were compared by using the generalized estimating equation method. The inter-reader agreement was evaluated using the Cohen $\kappa$ coefficient. The $\kappa$ values were interpreted as follows: poor, 0.00–0.20; fair, 0.21–0.40; moderate, 0.41–0.60; good, 0.61–0.80; and excellent, 0.81–1.00. Statistical analyses were performed using SAS software (version 9.4; SAS Inc., Cary, NC, USA). A P-value of <0.05 was considered statistically significant.
RESULTS

Characteristics of patients and lesions

A total of 273 patients (mean age, 57.3 years; 188 men and 85 women) with 352 lesions were selected for the final statistical analysis (Fig. 1). The characteristics of the patients and lesions are presented in Table 1. The study consisted of 71 patients with 86 lesions who underwent ECA-MRI, and 202 patients with 266 lesions who underwent HBA-MRI. No statistically significant differences in sex, age, presence of liver cirrhosis, lesion size, proportions of categories according to LI-RADS 2018 and KLCA-NCC 2018, and final diagnosis were found between ECA-MRI and HBA-MRI groups. Hepatitis B (89.8%) was the predominant etiology of liver disease. Two hundred eighteen (79.9%) patients had one lesion; 37 (13.6%) had two lesions; and 18 (6.5%) had three or more lesions. The 352 lesions included 263 (74.7%) HCCs, 37 (10.5%) non-HCC malignancies, and 52 (14.8%) benign lesions.

LR-5 of LI-RADS 2018 versus definite HCC of KLCA-NCC 2018

The per-lesion diagnostic performances for LR-5 of LI-RADS 2018 and definite HCC of KLCA-NCC 2018 are demonstrated in Table 2.

On ECA-MRI, the sensitivity and specificity of LR-5 of LI-RADS 2018 versus definite HCC of KLCA-NCC 2018 were not significantly different (75.8% vs. 69.4%, P=0.095 and 95.8% vs. 95.8%, P>0.999) for the entire cohort. In both subgroups according to the size of lesions (10–19 mm and ≥20 mm), there were no significantly differences in the sensitivities and specificities between LR-5 and definite HCC (all, P>0.05).

On HBA-MRI, the sensitivity was significantly higher for definite HCC of KLCA-NCC 2018 (79.1% vs. 68.2%, P<0.001) than for LR-5 of LI-RADS 2018 without a significant difference in specificity (93.9% vs. 95.4%, P=0.314) for all lesions. When lesions were stratified according to the size (10–19 mm and ≥20 mm), the sensitivities of definite HCC were significantly higher than those of LR-5 (10–19 mm: 75.8% vs. 51.6%, P<0.001; and ≥20 mm: 80.6% vs. 75.5%, P=0.017), but the specificities were not significantly different (all, P>0.05), similar to the results obtained for all lesions (Figs. 2, 3).

There were 27 lesions with threshold growth (24 HCCs and three non-HCC malignancies) according to LI-RADS 2018. By using only threshold growth (one of the major imaging features of LI-RADS 2018) with AP hyperenhancement, two additional HCCs were diagnosed as LR-5.

LR-5/4 of LI-RADS 2018 versus definite/probable HCC of KLCA-NCC 2018

The per-lesion diagnostic performances of LR-5/4 of LI-RADS 2018 and definite HCC of KLCA-NCC 2018 were not significantly different (75.8% vs. 69.4%, P=0.095 and 95.8% vs. 95.8%, P>0.999) for the entire cohort. In both subgroups according to the size of lesions (10–19 mm and ≥20 mm), there were no significantly differences in the sensitivities and specificities between LR-5 and definite HCC (all, P>0.05).

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LR-5/4 of LI-RADS 2018 versus definite/probable HCC of KLCA-NCC 2018

The per-lesion diagnostic performances of LR-5/4 of LI-RADS 2018 and definite HCC of KLCA-NCC 2018 were not significantly different (75.8% vs. 69.4%, P=0.095 and 95.8% vs. 95.8%, P>0.999) for the entire cohort. In both subgroups according to the size of lesions (10–19 mm and ≥20 mm), there were no significantly differences in the sensitivities and specificities between LR-5 and definite HCC (all, P>0.05).

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Table 1. Characteristics of 273 patients and 352 lesions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>ECA-MRI</th>
<th>HBA-MRI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>273</td>
<td>71</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>Sex, male</td>
<td></td>
<td></td>
<td></td>
<td>0.456</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>57.3±9.5</td>
<td>57.4±9.9</td>
<td>57.3±9.4</td>
<td>0.950</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>245 (89.8)</td>
<td>60 (84.5)</td>
<td>185 (91.6)</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>11 (4.0)</td>
<td>7 (9.9)</td>
<td>4 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>17 (6.2)</td>
<td>4 (5.6)</td>
<td>13 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>150 (54.9)</td>
<td>39 (54.9)</td>
<td>111 (55.0)</td>
<td></td>
</tr>
<tr>
<td>Lesions</td>
<td>352</td>
<td>86</td>
<td>266</td>
<td></td>
</tr>
<tr>
<td>Size (mm)†</td>
<td>24 (15–34)</td>
<td>25 (19–32)</td>
<td>24 (15–35)</td>
<td>0.416</td>
</tr>
<tr>
<td>Size subgroup</td>
<td></td>
<td></td>
<td></td>
<td>0.307</td>
</tr>
<tr>
<td>10–19 mm</td>
<td>132 (37.5)</td>
<td>28 (32.6)</td>
<td>104 (39.1)</td>
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<tr>
<td>≥20 mm</td>
<td>220 (62.5)</td>
<td>58 (67.4)</td>
<td>162 (60.9)</td>
<td></td>
</tr>
<tr>
<td>Categorization according to LI-RADS 2018</td>
<td></td>
<td></td>
<td></td>
<td>0.111</td>
</tr>
<tr>
<td>LR-2</td>
<td>30 (8.5)</td>
<td>7 (8.1)</td>
<td>23 (8.7)</td>
<td></td>
</tr>
<tr>
<td>LR-3</td>
<td>14 (4.0)</td>
<td>7 (8.1)</td>
<td>7 (2.6)</td>
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<tr>
<td>LR-4</td>
<td>59 (16.8)</td>
<td>9 (10.5)</td>
<td>50 (18.8)</td>
<td></td>
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<tr>
<td>LR-5</td>
<td>188 (53.4)</td>
<td>48 (55.8)</td>
<td>140 (52.6)</td>
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<tr>
<td>LR-M</td>
<td>59 (16.8)</td>
<td>14 (16.3)</td>
<td>45 (16.9)</td>
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<tr>
<td>LR-TIV</td>
<td>2 (0.5)</td>
<td>1 (1.2)</td>
<td>1 (0.4)</td>
<td></td>
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<tr>
<td>Categorization according to KLCA-NCC 2018</td>
<td></td>
<td></td>
<td></td>
<td>0.135</td>
</tr>
<tr>
<td>Benign</td>
<td>31 (8.8)</td>
<td>11 (12.8)</td>
<td>20 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>31 (8.8)</td>
<td>7 (8.1)</td>
<td>24 (9.0)</td>
<td></td>
</tr>
<tr>
<td>Probable HCC</td>
<td>24 (6.8)</td>
<td>10 (11.6)</td>
<td>14 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Definite HCC</td>
<td>207 (58.8)</td>
<td>44 (51.2)</td>
<td>163 (61.3)</td>
<td></td>
</tr>
<tr>
<td>Targetoid appearance</td>
<td>59 (16.8)</td>
<td>14 (16.3)</td>
<td>45 (16.9)</td>
<td></td>
</tr>
<tr>
<td>Final diagnosis</td>
<td></td>
<td></td>
<td></td>
<td>0.051</td>
</tr>
<tr>
<td>HCC</td>
<td>263 (74.7)</td>
<td>62 (72.1)</td>
<td>201 (75.6)</td>
<td></td>
</tr>
<tr>
<td>Non-HCC malignancy</td>
<td>37 (10.5)</td>
<td>9 (10.5)</td>
<td>28 (10.5)</td>
<td></td>
</tr>
<tr>
<td>chHCC-CCA</td>
<td>23 (6.6)</td>
<td>5 (5.8)</td>
<td>18 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Intrahepatic cholangiocarcinoma</td>
<td>9 (2.5)</td>
<td>3 (3.5)</td>
<td>6 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>1 (0.3)</td>
<td>1 (1.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>4 (1.1)</td>
<td>0 (0.0)</td>
<td>4 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Benign lesion</td>
<td>52 (14.8)</td>
<td>15 (17.4)</td>
<td>37 (13.9)</td>
<td></td>
</tr>
<tr>
<td>Hemangioma</td>
<td>14 (4.0)</td>
<td>4 (4.6)</td>
<td>10 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Focal nodular hyperplasia-like nodule</td>
<td>8 (2.3)</td>
<td>6 (7.0)</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Regenerative nodule</td>
<td>17 (4.8)</td>
<td>3 (3.5)</td>
<td>14 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Dysplastic nodule</td>
<td>13 (3.7)</td>
<td>2 (2.3)</td>
<td>11 (4.1)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation, median (interquartile), or number (%).
ECA, extracellular contrast agent; MRI, magnetic resonance imaging; HBA, hepatobiliary agent; LI-RADS, Liver Imaging Reporting and Data System; KLCA, Korean Liver Cancer Association; HCC, hepatocellular carcinoma; chHCC-CCA, combined hepatocellular carcinoma-cholangiocarcinoma.
*Compared by using the two-sample t test.
†Compared by using Mann-Whitney U test.
2018 and definite/probable HCC of KLCA-NCC 2018 are summarized in Table 3.

On ECA-MRI, no significant differences in the sensitivity and specificity were observed between LR-5/4 of LI-RADS 2018 and definite/probable HCC of KLCA-NCC 2018 (87.1% vs. 83.9%, P = 0.313 and 87.5% vs. 91.7%, P = 0.307) for the entire cohort. When lesions were stratified according to the size (10–19 mm and ≥20 mm), the sensitivities and specificities of LR-5/4 and definite/probable HCC were not significantly different for both subgroups (all, P > 0.05).

On HBA-MRI, definite/probable HCC of KLCA-NCC 2018 showed significantly higher specificity (92.3% vs. 80.0%, P = 0.003) than LR-5/4 of LI-RADS 2018 for diagnosing HCC without a statistically significant difference in sensitivity (85.6% vs. 88.1%, P = 0.057) for all lesions. The specificity of definite/probable HCC for 10–19 mm-sized lesions was significantly higher than that of LR-5/4 (92.9% vs. 73.8%, P = 0.002), but the sensitivities for 10–19 mm-sized lesions and ≥20 mm-sized lesions were not significantly different (all, P > 0.05).

### False-positive diagnoses for HCC

There were four false-positive diagnoses in LR-5 of LI-RADS 2018 and five false-positive diagnoses in definite HCC of KLCA-NCC 2018. All false-positive results were combined hepatocellular carcinoma-cholangiocarcinoma (cHCC-CCA) (Fig. 4, Supplementary Table 4).

In LR-5/4 of LI-RADS 2018, the false-positive results were eight non-HCC malignancies (six HCC-CCAs, one hepatoblastoma, and one metastasis) and eight benign lesions (two foci nodular hyperplasia-like nodules and six dysplastic nodules). In definite/probable HCC of KLCA-NCC 2018, the false-positive diagnoses were six cHCC-CCAs and one hepatoblastoma (Supplementary Table 5).

#### Subgroup analysis in patients with hepatitis B

Supplementary Table 6 and 7 present the subgroup analysis results in patients with hepatitis B. The results of patients with hep-
On ECA-MRI, the sensitivity and specificity of LI-RADS 2018 and KLCA-NCC 2018 were not significantly different (LR-5 vs. definite HCC: 75.0% vs. 67.9%, $P=0.094$ and 93.3% vs. 93.3%, $P>0.999$; LR-5/4 vs. definite/probable HCC: 87.5% vs. 83.9%, $P=0.313$ and 86.7% vs. 86.7%, $P>0.999$).

On HBA-MRI, definite HCC of KLCA-NCC 2018 showed significantly higher sensitivity (79.1% vs. 68.5%, $P<0.001$) than LR-5 of LI-RADS 2018 without a significant difference in specificity (94.9% vs. 96.6%, $P=0.313$). Definite/probable HCC of KLCA-NCC 2018 had higher specificity (93.2% vs. 81.4%, $P=0.005$) than LR-5/4 of LI-RADS 2018. The sensitivity was lower for definite/probable HCC than for LR-5/4 without statistical significance (85.0% vs. 87.7%, $P=0.056$).

### Inter-reader agreement

The inter-reader agreement for the categorization of lesions ac-

Figure 2. HCC in a 53-year-old man with chronic hepatitis B. On the arterial (A), portal venous (B), and hepatobiliary phase (C) images, after administration of hepatobiliary agent, a 39-mm liver mass (arrows) showed arterial phase hyperenhancement without washout in the portal venous phase, while showing hypointensity in the hepatobiliary phase. The mass was categorized as LR-4 by LI-RADS 2018, but classified as definite HCC by KLCA-NCC 2018. HCC, hepatocellular carcinoma; LI-RADS, Liver Imaging Reporting and Data System; KLCA-NCC, Korean Liver Cancer Association-National Cancer Center.

Figure 3. HCC in a 60-year-old woman with cirrhosis related to hepatitis B. On the arterial (A), portal venous (B), and hepatobiliary phase (C) images after administration of hepatobiliary agent, a 36-mm liver mass (arrows) showed arterial phase hyperenhancement without washout but with enhancing capsule in the portal venous phase, and hyperintensity in the hepatobiliary phase. On T2-weighted image (D), the mass demonstrated mild-moderate T2 hyperintensity. The mass was categorized as LR-5 by LI-RADS 2018, but classified as probable HCC by KLCA-NCC 2018. HCC, hepatocellular carcinoma; LI-RADS, Liver Imaging Reporting and Data System; KLCA-NCC, Korean Liver Cancer Association-National Cancer Center.
**Table 3.** Diagnostic performances of LR-5/4 of LI-RADS 2018 and definite/probable HCC of KLCA-NCC 2018 for diagnosing HCC on ECA-MRI and HBA-MRI

<table>
<thead>
<tr>
<th></th>
<th>LR-5/4 of LI-RADS 2018 (95% CI)</th>
<th>Definite/probable HCC of KLCA-NCC 2018 (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECA-MRI all (n=86)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.1 (78.8–95.4)</td>
<td>83.9 (74.7–93.0)</td>
<td>0.313</td>
</tr>
<tr>
<td>Specificity</td>
<td>87.5 (74.3–100.0)</td>
<td>91.7 (80.6–100.0)</td>
<td>0.307</td>
</tr>
<tr>
<td>ECA-MRI 10–19 mm (n=28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>94.1 (82.9–100.0)</td>
<td>76.5 (56.3–96.6)</td>
<td>0.056</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0 (100.0–100.0)</td>
<td>100.0 (100.0–100.0)</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>ECA-MRI ≥20 mm (n=58)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>84.4 (73.9–95.0)</td>
<td>86.7 (76.7–96.6)</td>
<td>0.312</td>
</tr>
<tr>
<td>Specificity</td>
<td>76.9 (54.0–99.8)</td>
<td>84.6 (65.0–100.0)</td>
<td>0.298</td>
</tr>
<tr>
<td>HBA-MRI all (n=266)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>88.1 (83.6–92.5)</td>
<td>85.6 (80.7–90.4)</td>
<td>0.057</td>
</tr>
<tr>
<td>Specificity</td>
<td>80.0 (70.3–89.7)</td>
<td>92.3 (85.8–98.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>HBA-MRI 10–19 mm (n=104)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.1 (78.8–95.4)</td>
<td>82.3 (72.8–91.8)</td>
<td>0.076</td>
</tr>
<tr>
<td>Specificity</td>
<td>73.8 (60.5–87.1)</td>
<td>92.9 (85.1–100.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>HBA-MRI ≥20 mm (n=162)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>88.5 (83.2–93.8)</td>
<td>87.1 (81.5–92.6)</td>
<td>0.316</td>
</tr>
<tr>
<td>Specificity</td>
<td>91.3 (79.8–100.0)</td>
<td>91.3 (79.8–100.0)</td>
<td>&gt;0.999</td>
</tr>
</tbody>
</table>

LI-RADS, Liver Imaging Reporting and Data System; HCC, hepatocellular carcinoma; KLCA-NCC, Korean Liver Cancer Association-National Cancer Center; ECA, extracellular contrast agent; MRI, magnetic resonance imaging; HBA, hepatobiliary agent; CI, confidence interval.

*P-values between LR-5/4 of LI-RADS 2018 and definite/probable HCC of KLCA-NCC 2018 by using the generalized estimating equation method.

**Figure 4.** cHCC-CCA in a 62-year-old man with chronic hepatitis B. On the arterial (A), portal venous (B), and delayed phase (C) images, after administration of extracellular contrast agent, a 26-mm liver mass (arrows) showed arterial phase hyperenhancement with washout and enhancing capsule in the portal venous and delayed phases. The mass was categorized as LR-5 by LI-RADS 2018, and classified as definite HCC by KLCA-NCC 2018. However, it was diagnosed as cHCC-CCA by surgical resection. cHCC-CCA, combined hepatocellular carcinoma-cholangiocarcinoma; LI-RADS, Liver Imaging Reporting and Data System; KLCA-NCC, Korean Liver Cancer Association-National Cancer Center.
DISCUSSION

Our study showed that LI-RADS 2018 and KLCA-NCC 2018 had comparable diagnostic performances on ECA-MRI. On HBA-MRI, definite HCC of KLCA-NCC 2018 showed better sensitivity without a significant change in specificity, compared to LR-5 of LI-RADS 2018. Definite/probable HCC of KLCA-NCC 2018 provided higher specificity than LR-5/4 of LI-RADS 2018 when using HBA-MRI.

Our study indicates that KLCA-NCC 2018 may provide better diagnostic performance than LI-RADS 2018 to diagnose HCC with HBA-MRI, but not with ECA-MRI. The discrepancy may be attributed to the different definitions of washout appearance between the two systems. LI-RADS 2018 states that washout should be determined in the PVP only for HBA-MRI, whereas KLCA-NCC 2018 defines washout not only in the PVP but also in the TP and HBP. In our study, on HBA-MRI, the use of washout appearance defined by KLCA-NCC 2018 enabled the diagnosis of 23 additional HCC lesions compared to that defined by LI-RADS 2018, which resulted in increased sensitivity for definite HCC of KLCA-NCC 2018. This result is consistent with the results of recent studies that reported improved sensitivity by using HBP hypointensity as an alternative to washout on HBA-MRI. Nevertheless, definite HCC of KLCA-NCC 2018 did not significantly reduce its specificity compared with LR-5 of LI-RADS 2018. The diagnostic criteria for HCC according to KLCA-NCC 2018 may prevent significant loss in specificity by excluding hemangiomas or other malignancies, based on ancillary features favoring benignity such as marked T2 hyperintensity or targetoid appearance on DWI or contrast-enhanced sequences prior to the application of major imaging features. The use of a sequential algorithm with stepwise consideration in KLCA-NCC 2018 as in LI-RADS 2018 seems to help maintain a high specificity for the diagnosis of HCC.

LI-RADS 2018 and KLCA-NCC 2018 are unique compared to other noninvasive imaging criteria in that they adapt non-binary decision algorithms and incorporate ancillary imaging features to modulate the likelihood of HCC. They allow categories for probably HCC (designated as LR-4 of LI-RADS 2018 and probable HCC of KLCA-NCC 2018) with different combinations of major and ancillary imaging features. Observations that do not meet stringent LR-5 criteria of LI-RADS or lesions with some but not all of the major imaging features of KLCA-NCC 2018 criteria can be appropriately categorized as LR-4 of LI-RADS 2018 or probable HCC of KLCA-NCC 2018. While an observation can be upgraded from LR-3 to LR-4 with any of the ancillary features favoring malignancy in LI-RADS 2018, a lesion classified as probable HCC in KLCA-NCC 2018 criteria requires at least one item from each of the two categories of ancillary imaging features (favoring malignancy in general and favoring HCC in particular). This difference may contribute to a statistically no significant difference in sensitivity but significantly higher specificity in definite/probable HCC of KLCA-NCC 2018 as compared with LR-5/4 of LI-RADS 2018. In the present study, LR-5/4 resulted in an unacceptably low specificity, which is consistent with some recent studies and a recent meta-analysis. Meanwhile, definite/probable HCC of KLCA-NCC 2018 maintained high specificity (>90%). In definite/probable HCC of KLCA-NCC 2018, false positive results were mostly due to chCC-CCA, defined as primary liver carcinoma with both hepatocytic and cholangiocytic differentiation. Several studies have also reported that some chCC-CCAs might not be distinguishable from HCCs based on imaging features.

Our study has strength by comparing the diagnostic systems for the noninvasive diagnosis of HCC and identifying proper imaging criteria according to MRI contrast agents. This study indicates that KLCA-NCC 2018 may be more appropriate diagnostic criteria for HCC when using HBA-MRI. A recent prospective study comparing ECA-MRI and HBA-MRI intrinsically for the diagnosis of HCC has shown that the current LR-5 criteria of LI-RADS limited the sensitivity on HBA-MRI. In our study, LR-5 of LI-RADS 2018 also revealed suboptimal sensitivity on HBA-MRI, whereas definite HCC of KLCA-NCC 2018 showed higher sensitivity when using HBA-MRI. In particular, there was a greater difference in the sensitivities of <10–19 mm lesions on HBA-MRI between LI-RADS and the KLCA-NCC 2018 guidelines. The diagnostic criteria for HCC need to be adjusted according to MRI contrast agent. In addition, imaging criteria reflect geographic differences in clinical environment and treatment strategies. In Korea, early treatment with locoregional therapies is widely used for HCC, thus, the use of KLCA-NCC 2018 diagnostic criteria may provide better sensitivity without compromise in specificity and help detection and early diagnosis of small HCC when using HBA-MRI.

This study has several limitations. First, the retrospective study design at a single center may have introduced an inevitable selection bias. Second, the predominance of patients with chronic hepatitis B in our study population may limit the generalizability of
the results to other geographic populations with different etiologies of HCC. Additional prospective multicenter studies that include patients with various etiologies of liver disease are required to validate our results. Third, we used the reference standard for the final diagnosis of benign lesion not based on a pathological diagnosis alone but on a composite clinical reference standard. However, pathologic confirmation for highly suspected benign lesions is not recommended in clinical practice, and application of a strict standard of reference (only pathology) for benign lesions may have resulted in a confirmation bias. Finally, the blinded readers had participated in imaging diagnoses in their daily practice; thus, recall bias might have occurred.

In conclusion, LI-RADS 2018 and KLCA-NCC 2018 show comparable diagnostic performances on ECA-MRI. On HBA-MRI, definite HCC of KLCA-NCC 2018 provides better sensitivity and accuracy than LR-5 category of LI-RADS 2018 and definite/probable HCC of KLCA-NCC 2018 reveals higher specificity than LR-5/4 of LI-RADS 2018 for the noninvasive diagnosis of HCC.

Authors’ contribution
All authors contributed to the interpretation of results, critical revision of the manuscript, and approved the final manuscript.

-Guarantor of the integrity of the entire study: M.-J. Kim
-Study concept and design: S. Lee, S.-S. Kim, and M.-J. Kim
-Acquisition of data: S. Lee, S.-S. Kim, D.-R. Chang, and M.-J. Kim
-Statistical analysis: H. Kim
-Drafting of the manuscript: S. Lee and M.-J. Kim

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Conflicts of Interest
The authors have no conflicts to disclose.

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

REFERENCES
Entecavir+tenofovir vs. lamivudine/telbivudine+ adefovir in chronic hepatitis B patients with prior suboptimal response

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INTRODUCTION

Patients with chronic hepatitis B (CHB) and continuously high levels of serum hepatitis B virus (HBV) DNA have an increased risk for progression of hepatic fibrosis and development of hepatocellular carcinoma (HCC). Current treatments for CHB aim to reduce the risk of hepatic events by providing complete virologic suppression. The introduction of nucleotide/nucleoside analogues (NUCs), which block the reverse transcription of HBV polymerase, has markedly improved the prognosis of these patients. However, drug resistance remains a clinical challenge when using antiviral therapies for CHB. The widespread use of antiviral agents with low genetic barriers to resistance, such as lamivudine (LAM), adefovir (ADV), telbivudine (LdT), and clevudine (approved in South Korea), has led to increased drug resistance. Moreover, patients with LAM-resistant hepatitis B virus (LAM-resistant HBV) infections often have suboptimal responses to LAM/LdT+ADV rescue therapy.

Background/Aims: Suboptimal responses to lamivudine or telbivudine plus adefovir (LAM/LdT+ADV) rescue therapy are common in patients with LAM-resistant hepatitis B virus (HBV) infections. We compared patients switched to entecavir plus tenofovir (ETV+TDF) to those maintained on LAM/LdT+ADV.

Methods: This prospective randomized controlled trial examined 91 patients whose serum HBV DNA levels were greater than 60 IU/mL after at least 24 weeks of treatment with LAM/LdT+ADV for LAM-resistant HBV. Patients were randomly assigned to receive a new treatment (ETV+TDF, n=45) or to continue the same treatment (LAM/LdT+ADV, n=46) for 48 weeks. Patients with baseline ADV resistance were excluded.

Results: Compared to LAM/LdT+ADV group, ETV+TDF group had more patients with a virologic response (42/45 [93.33%] vs. 3/46 [6.52%], P<0.001) and had a greater mean reduction in serum HBV DNA level from baseline (-4.16 vs. -0.37 log10 IU/mL, P<0.001). Multivariate analysis indicated that high baseline HBV DNA level (P=0.005) and LAM/LdT+ADV maintenance therapy (P=0.001) were negatively associated with virologic response. At week 48, additional ADV- or ETV-associated mutations were cleared in ETV+TDF group, but such mutations were present in 4.3% of patients in LAM/LdT+ADV group (P=0.106). The two groups had similar rates of adverse events.

Conclusions: ETV+TDF combination treatment led to a significantly higher rate of virologic response compared to LAM/LdT+ADV combination treatment in patients with LAM-resistant HBV who had suboptimal responses to LAM/LdT+ADV regardless of HBV genotypic resistance profile (NCT01597934). (Clin Mol Hepatol 2020;26:352-363)

Keywords: Entecavir; Tenofovir; Lamivudine; Antiviral drug resistance; Adefovir

Study Highlights
This study showed that ETV+TDF led to a significantly higher rate of virologic response compared to LAM/LdT+ADV in patients with LAM-resistant HBV who had suboptimal responses to LAM/LdT+ADV regardless of the HBV genotypic resistance profile. Continuation of LAM/LdT+ADV afforded very little antiviral benefit, and increased the risk of RT mutations that confer drug resistance. The two treatment regimens had similar safety profiles.

Abbreviations:
ADV, adefovir; AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LAM, lamivudine; LdT, telbivudine; NUCs, nucleotide/nucleoside analogues; PCR, polymerase chain reaction; RT, reverse transcriptase; TDF, tenofovir; ULN, upper normal limit; YMDD, tyrosine-methionine-aspartic acid-aspartic acid

*These authors are equally contributed to this work as the corresponding author.
Korea), as initial treatment is one of the main causes of the high prevalence of genotypic resistance to NUCs among patients with CHB in Asian countries. For example, patients taking LAM, the first approved oral nucleoside, have a 65% incidence of drug resistance after 5 years of treatment. Before the introduction of tenofovir (TDF) and entecavir (ETV), ADV was the only available rescue therapy for patients with LAM resistance. However, sequential ADV monotherapy after the development of LAM resistance fails to achieve adequate virologic suppression in up to 25% of patients, and can also cause the development of genotypic resistance. Several studies reported that a substantial proportion of patients who were treated with LAM+ADV combination therapy developed persistently inadequate or suboptimal virologic responses, and that mutations in the tyrosine-methionine-aspartic acid-aspartic acid (YMDD) motif persisted despite rescue combination therapy. A suboptimal response to antiviral therapy can increase the risk of developing resistance to multiple NUCs, and also increase the risk of end-stage liver disease and HCC. Therefore, current guidelines suggest that the ideal treatment for CHB is to reduce the serum HBV DNA level to below the detection limit of real-time polymerase chain reaction (PCR).

There is little consensus regarding the most effective antiviral therapy for patients with CHB who have suboptimal responses after LAM+ADV combination therapy. Before the approval of TDF, ETV+ADV was the most potent combination therapy for patients with CHB who had suboptimal responses to LAM+ADV. After its approval, TDF became an important and potent NUC used in antiviral regimens against CHB. In particular, TDF has high antiviral efficacy in patients with LAM resistance. However, in patients who have failed to respond to LAM+ADV, previous research suggested that the efficacy of ETV or TDF monotherapy was inferior to that achieved in treatment-naïve patients. This emphasizes the need to identify the most effective combination therapy for treatment of multidrug-refractory CHB. Moreover, as patients with CHB require long-term antiviral therapy, they may develop resistance to treatments, even TDF-containing regimens. Combination treatment may be a better option than monotherapy to prevent further resistance in patients with LAM-resistant HBV. A recent retrospective study in South Korea showed the superior efficacy of ETV+TDF compared to ETV+ADV in patients with LAM-resistant HBV. No previous prospective studies have compared the efficacy of TDF+ETV with LAM/LdT+ADV in LAM-resistant patients who had suboptimal responses to LAM+ADV combination therapy. The present prospective study of patients with CHB with resistance to LAM or LdT, who showed suboptimal responses to antiviral combination rescue therapy (LAM+ADV or LdT+ADV), compared the efficacy and safety of switching to TDF+ETV rather than maintaining LAM/LdT+ADV.

**MATERIALS AND METHODS**

**Study design**

This study was a randomized, open-label, prospective, multicenter trial of patients with infections by LAM- or LdT-resistant HBV (YMDD mutation) who had suboptimal responses to antiviral combination rescue therapy (LAM+ADV or LdT+ADV), and were receiving this therapy for at least 24 weeks. Patients were subjected to block randomization (1:1 ratio) and assigned to two treatment arms: 1) ETV (1.0 mg) plus TDF (300 mg) once daily or 2) LAM (100 mg) plus ADV (10 mg) or LdT 600 mg plus ADV (10 mg) once daily. The primary endpoint was measured at week 48. At week 48, further treatment with commercially available therapies performed was at the discretion of the investigator. During treatment, any patient who developed virologic breakthrough and showed alanine aminotransferase (ALT) flare (10-fold above the upper normal limit [ULN] of 40 IU/mL) or liver decompensation was dropped out of the study and transitioned to commercially available antiviral therapy.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki 1975 (revised in 2000) and the regulatory requirements of all participating countries. Institutional approval was obtained at all clinical sites, and written informed consent was provided by all study participants. This study was registered at ClinicalTrials.gov under number NCT01597934 (https://clinicaltrials.gov/ct2/show/NCT01597934).

**Patients**

Patients with CHB, all of whom had detectable hepatitis B surface antigen (HBsAg) at screening and at 24 weeks or more before screening, were recruited from six hospital clinics in South Korea. Eligible patients were male or female, aged 20 years or older, hepatitis B e-antigen (HBeAg)-positive or -negative, and had compensated liver function (Child-Pugh class A). In addition, all patients had genotypic resistance to LAM or LdT (YMDD mutation) and were classified as suboptimal responders, defined as a >1 log, IU/mL reduction in HBV DNA but with detectable HBV DNA (>60 IU/mL) after combination treatment with LAM.
(100 mg/day) plus ADV (10 mg/day) for at least 24 weeks that was ongoing at the time of randomization.

Patients with any of the following characteristics were excluded: history of genotypic resistance to ADV; ALT level more than 10-fold above the ULN; most recent treatment that was not LAM+ADV or LdT+ADV; coinfection with HCV or HIV; pregnant or lactating; long-term use of an immunosuppressant or agent associated with high risk of hepatic/renal toxicity; history of liver transplantation or planning for liver transplantation; diagnosis of a malignant tumor and receiving chemotherapy; history of HCC or evidence of HCC; renal insufficiency (creatinine clearance rate below 50 mL/min based on the Cockcroft-Gault equation); liver disease other than CHB (e.g., hemochromatosis, Wilson’s disease, alcoholic liver disease, nonalcoholic fatty liver disease); and history of hypersensitivity to a study drug.

Outcome analyses

The primary efficacy endpoint was virologic response, defined as an HBV DNA level below 60 IU/mL (approximately 300 copies/mL) based on real-time PCR measurements at week 48.

The secondary efficacy endpoints were: 1) HBV DNA level below 60 IU/mL at weeks 12, 24, and 36; 2) ALT normalization (below the ULN); 3) mean HBV DNA level at weeks 12, 24, 36, and 48; and 4) HBeAg loss, HBeAg seroconversion (among HBeAg positive patients), and HBSAg loss at weeks 24 and 48. Surveillance of HBV antiviral drug resistance was performed on all baseline samples and at weeks 12, 24, 36, and 48 if there was detectable HBV DNA.

After randomization, patients were evaluated at baseline and at weeks 12, 24, 36, and 48. At each visit, vital signs were recorded, physical examination was performed, and adverse events were recorded. Efficacy analyses were based on plasma HBV DNA level, liver biochemistry, and hematology (all measured at baseline and at weeks 12, 24, 36, and 48). Serological measurements of HBsAg, anti-HBs antibody, HBeAg, and anti-HBe antibody were performed at weeks 24 and 48 using a radioimmunoassay (Abbott Laboratories, Abbott Park, IL, USA).

Routine biochemical tests (ALT, aspartate aminotransferase [AST], albumin, total bilirubin, and serum creatinine) were performed using a Sequential Multiple Autoanalyzer. HBV DNA was measured using real-time PCR assay on a Cobas TaqMan 48 Analyzer (Roche Molecular Diagnostics, Branchburg, NJ, USA), which had a limit of detection of 20 IU/mL. Genotypic resistance was determined using restriction fragment mass polymorphism.

Adverse events (AEs) and safety-related clinical laboratory tests were recorded at every visit. All patients with AEs that led to discontinuation were recorded up to week 48.

Statistical analysis

Efficacy analyses were performed based on patients who received at least one dose of the study medication (modified intent-to-treat analysis). Discontinuation of treatment prior to week 48 was considered treatment failure for the primary endpoint. Since there were no clinical data on ETV+TDF combination treatment at the time of this study, the projected response rates were based on a previous study (4% for LAM+ADV and 29% for ETV+ADV). Comparison of the number of patients who achieved the primary endpoint used two-sided Fisher’s exact test. A sample size of 104 randomized patients (52 patients per treatment arm) was estimated to provide at least 90% power to detect a difference of up to 25% between the two groups, based on Fisher’s exact test with a significance level of 0.05 and assuming a dropout rate of 20% over 1 year.

Variables were expressed as means with standard deviations or numbers and percentages. Between-group comparisons of continuous variables were determined using an independent t-test. Categorical variables were compared using either chi-square test or Fisher’s exact test, as appropriate. Cumulative virologic response during treatment was calculated using the Kaplan-Meier method, and curves were compared using a log-rank test. Univariate and multivariate analyses were performed using logistic regression to identify the factors associated with virologic response at week 48. Factors with P-values <0.2 in univariate analysis, along with clinical factors found to be important in previous studies, were included in the multivariate analysis. A P-value below 0.05 was considered significant. All data were analyzed using SPSS (version 20.0; IBM, Somers, NY, USA).

RESULTS

Patient population

We initially randomized 116 HBV patients, but excluded 25 patients who were deemed ineligible. As a result, we ultimately enrolled 45 patients in ETV+TDF group and 46 patients in LAM/LdT+ADV group (Fig. 1). At the end of this study (week 48), 89 patients received commercially available anti-HBV therapies; two
patients in LAM/LdT+ADV group were lost to follow-up. The two
groups had similar baseline characteristics (Table 1). More specifi-
cally, the two groups had similar rates of liver cirrhosis (13.33% vs.
19.57%, P=0.423), HBeAg positivity (88.89% vs. 95.65%,
P=0.266), HBV DNA level (4.36 vs. 4.08 log_{10} IU/mL, P=0.228),
and ALT level (27 vs. 32 U/L, P=0.422). The two groups also had
no significant differences in prior antiviral treatment regimens
(Table 2) and genotypic resistance profiles (Table 3).

![Study design. LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir.](https://doi.org/10.3350/cmh.2019.0044n)

**Figure 1.** Study design. LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir.

**Table 1.** Baseline characteristics of the two study groups

<table>
<thead>
<tr>
<th></th>
<th>LAM/LdT+ADV (n=46)</th>
<th>ETV+TDF (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.0±10.5</td>
<td>50.0±11.0</td>
<td>0.978</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>28 (60.9)</td>
<td>31 (68.9)</td>
<td>0.512</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>9 (19.5)</td>
<td>6 (13.3)</td>
<td>0.574</td>
</tr>
<tr>
<td>Duration of prior NUC treatment (weeks)</td>
<td>307.9±116.8</td>
<td>327.7±113.4</td>
<td>0.536</td>
</tr>
<tr>
<td>Duration of ADV-based treatment (weeks)</td>
<td>166.8±82.3</td>
<td>173.1±85.3</td>
<td>0.934</td>
</tr>
<tr>
<td>Prior use of ETV</td>
<td>11 (23.9)</td>
<td>16 (35.6)</td>
<td>0.257</td>
</tr>
<tr>
<td>Log_{10} HBV DNA (IU/mL)</td>
<td>4.0±0.7</td>
<td>4.3±0.9</td>
<td>0.228</td>
</tr>
<tr>
<td>HBeAg positive</td>
<td>43 (93.5)</td>
<td>40 (88.9)</td>
<td>0.485</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>32.0±22.0</td>
<td>27.0±17.4</td>
<td>0.422</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>28.0±12.7</td>
<td>23.0±8.3</td>
<td>0.285</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.7±0.3</td>
<td>0.7±0.2</td>
<td>0.952</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.6±0.2</td>
<td>4.6±0.3</td>
<td>0.634</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.3±0.3</td>
<td>9.3±0.3</td>
<td>0.850</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>3.4±0.7</td>
<td>3.4±0.5</td>
<td>0.613</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8±0.2</td>
<td>0.8±0.1</td>
<td>0.718</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>42.0±20.5</td>
<td>42.0±20.5</td>
<td>0.889</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%).
LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir; NUC, nucleos(t)ides; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, Estimated glomerular filtration rate.
Virologic response

The proportion of patients who achieved virologic response (HBV DNA level <60 IU/mL) at week 48 was significantly higher in ETV+TDF group than in LAM/LdT+ADV group (93.33% vs. 6.52%, P < 0.001). ETV+TDF group also had a significantly greater cumulative virologic response at week 12 (73.33% vs. 6.52%), week 24 (82.22% vs. 4.34%), week 36 (88.89% vs. 4.34%), and week 48 (93.33% vs. 6.52%) (log-rank-test: P <0.001 for all comparisons; Fig. 2). A total of 91.3% of patients who continued LAM/LdT+ADV treatment had virologic nonresponse (defined as a <1 log10 IU/mL reduction in HBV DNA concentration from baseline at week 24) at week 24, and 86.9% did not have a virologic response (HBV DNA level ≥60 IU/mL) at week 48. A total of 7% (3/45) of the patients who received ETV+TDF did not have a virologic response at week 48. These patients were all HBeAg-positive males who received previous ETV treatment. Their mean HBV DNA level was 5.93 log10 IU/mL at baseline, 4.63 log10 IU/mL at week 12, 4.31 log10 IU/mL at week 24, 3.97 log10 IU/mL at week 36, and 3.07 log10 IU/mL at week 48. A study of baseline mutations in reverse transcriptase (RT) indicated that one patient had rt180 (Met>Leu)+rt204(Val>Met)+rt184(Leu>Thr), one patient had rt204 (Ile>Met), and one patient had rt180 (Met>Leu)+rt204(Val>Met). These mutations were still present, and additional mutations did not develop until week 48. The mean time to

Table 2. Prior nucleos(t)ide treatment regimens in the two study groups

<table>
<thead>
<tr>
<th>Prior regimen</th>
<th>LAM/LdT+ADV</th>
<th>ETV+TDF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ETV use</td>
<td></td>
<td></td>
<td>0.499</td>
</tr>
<tr>
<td>LAM</td>
<td>21 (45.7)</td>
<td>13 (28.9)</td>
<td></td>
</tr>
<tr>
<td>LdT</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>CLV</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>LAM, ADV</td>
<td>10 (21.7)</td>
<td>9 (20)</td>
<td></td>
</tr>
<tr>
<td>CLV, ADV</td>
<td>1 (2.2)</td>
<td>3 (6.7)</td>
<td></td>
</tr>
<tr>
<td>LAM, CLV</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>LAM, ADV, LdT</td>
<td>1 (2.2)</td>
<td>2 (4.4)</td>
<td></td>
</tr>
<tr>
<td>ETV use</td>
<td>11 (23.9)</td>
<td>16 (35.6)</td>
<td></td>
</tr>
<tr>
<td>LAM, ETV</td>
<td>5 (10.9)</td>
<td>6 (13.3)</td>
<td></td>
</tr>
<tr>
<td>CLV, ETV</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>ADV, ETV</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>PegIFN, ETV</td>
<td>1 (2.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>LAM, ADV, ETV</td>
<td>5 (10.9)</td>
<td>5 (11.1)</td>
<td></td>
</tr>
<tr>
<td>LAM, ETV, LdT</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>ETV, ADV, LdT</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>LAM, ADV, ETV, LdT</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%).

LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir; CLV, clevudine; PegIFN, peginterferon.

Table 3. HBV reverse transcriptase mutations of patients in the two study groups

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>LAM/LdT+ADV</th>
<th>ETV+TDF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1 (2.2)</td>
<td>2 (4.4)</td>
<td>0.603</td>
</tr>
<tr>
<td>rt204(Ile→Met)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)</td>
<td>13 (28.3)</td>
<td>10 (22.2)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Ile→Met)</td>
<td>9 (19.6)</td>
<td>12 (26.7)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Ile→Met)+rt204(Val→Met)</td>
<td>2 (4.3)</td>
<td>5 (11.1)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt202(Gly→Ser)</td>
<td>1 (2.2)</td>
<td>2 (4.4)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt184(Leu→Thr)</td>
<td>1 (2.2)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt184(Ser/Met→Thr)</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt184(Leu→Thr)+rt202(Gly→Ser)</td>
<td>1 (2.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt184(Leu→Thr)+rt250(Val→Met)</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>rt180 (Met→Leu)+rt204(Val→Met)+rt184(Ile/Ala→Thr)+rt202(Gly→Ser)</td>
<td>1 (2.2)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%).

LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir; Ile, isoleucine; Met, methionine; Leu, leucine; Val, valine; Ser, serine; Thr, threonine; Gly, glycine.
virologic response was 18.33 weeks in ETV+TDF group and 43.13 weeks in LAM/LdT+ADV group.

Our analysis of the changes in viral load over time (Fig. 3) indicated that the mean log_{10} HBV DNA level (IU/mL) in the two groups were similar at baseline (4.08 vs. 4.36 IU/mL). However, TDF+ETV had significantly greater decreases at weeks 12, 24, 36, and 48 ($P<0.001$ for all comparisons).

Univariate and multivariate analyses were performed to identify the factors associated with virologic response at week 48 (Table 4). Factors assessed in univariate analyses included treatment method (LAM/LdT+ADV vs. TDF+ETV), age, sex, presence of liver cirrhosis, overall duration of previous NUC treatment, duration of previous ADV treatment, serum baseline HBV DNA concentration, HBeAg positivity, prior use of ETV, baseline resistance, and ALT level. Multivariate analysis showed that treatment with TDF+ETV and low baseline HBV DNA concentration were significantly and positively associated with virologic response.

We performed the same analyses separately for each treatment group (data not shown). Only the patients in LAM/LdT+ADV group who achieved virologic response had low baseline HBV DNA levels (<4 vs. $\geq 4$ log_{10} IU/mL: 21.1% [4/19] vs. 0% [0/26], $P=0.026$). Patients in ETV+TDF group were less likely to achieve a

![Figure 2. Cumulative virologic responses in the two groups. Solid line: TDF+ETV group; dotted line: LAM/LdT+ADV group. ETV, entecavir; TDF, tenofovir; LAM, lamivudine; LdT, telbivudine; ADV, adefovir.](image)

![Figure 3. Reduction of HBV DNA level (mean log_{10} IU/mL) in the two groups from baseline to week 48. Solid line: TDF+ETV group; dotted line: LAM/LdT+ADV group. HBV, hepatitis B virus; LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; TDF, tenofovir.](image)

### Table 4. Univariate and multivariate analyses of the association of clinical factors with virologic response at week 48

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>0.992</td>
<td>0.954–1.031</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>1.181</td>
<td>0.495–2.819</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>1.714</td>
<td>0.555–5.297</td>
</tr>
<tr>
<td>Duration of prior NUC treatment</td>
<td>0.995</td>
<td>0.981–1.010</td>
</tr>
<tr>
<td>Duration of ADV-based treatment</td>
<td>0.995</td>
<td>0.975–1.015</td>
</tr>
<tr>
<td>log_{10} HBV DNA</td>
<td>1.172</td>
<td>0.721–1.906</td>
</tr>
<tr>
<td>HBeAg positivity</td>
<td>6.450</td>
<td>0.744–55.948</td>
</tr>
<tr>
<td>ALT</td>
<td>1.017</td>
<td>0.993–1.041</td>
</tr>
<tr>
<td>Prior use of ETV</td>
<td>0.857</td>
<td>0.344–2.137</td>
</tr>
<tr>
<td>Treatment method, TDF+ETV vs. LAM/LdT+ADV</td>
<td>0.007</td>
<td>0.001–0.033</td>
</tr>
<tr>
<td>Baseline genotypic resistance</td>
<td>1.232</td>
<td>0.968–1.569</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; NUC, nucleos(t)ide; ADV, adefovir; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; ETV, entecavir; TDF, tenofovir; LAM, lamivudine; LdT, telbivudine.
virologic response if they had high baseline HBV DNA levels (<5 vs. \(\geq 5 \log_{10} \text{IU/mL}: 100\% [36/36] \text{ vs. } 66.7\% [6/9], P = 0.006\)). None of the other baseline clinical factors were associated with virologic response in this subgroup analysis.

**Resistance surveillance**

We collected serum samples from baseline to week 48 in all patients. For patients with detectable serum HBV DNA, we genotypically analyzed the samples for mutations in HBV RT that are associated with resistance to LAM, ADV, and ETV (Table 5). Among those with one or more RT mutations associated with ETV resistance at baseline, 16.6\% (1/6) in ETV+TDF group and 75\% (3/4) in LAM/LdT+ADV group retained these mutations until week 48 (\(P = 0.106\)). Among those who did not have RT mutations associated with ETV resistance at baseline, two of 39 patients in ETV+TDF group developed RT mutations associated with ETV resistance (one at week 12 and one at week 24). In LAM/LdT+ADV group, two of 42 patients developed mutations associated with ETV resistance (one at week 36 and one at week 48). Among patients with ETV resistance at baseline or during treatment, three patients in ETV+TDF group (one at baseline, one at week 12, and one at week 24) and two patients in LAM/LdT+ADV group (one at week 36, one at week 48) were ETV-naïve. However, in ETV+TDF group, complete suppression was achieved in all but one patient with baseline ETV resistance mutations in RT after 48 weeks of ETV+TDF treatment. In LAM/LdT+ADV group, virologic response could not be achieved in three of four patients who had baseline RT mutations associated with ETV resistance, and additional mutations associated with ETV resistance developed even when continuing LAM/LdT+ADV treatment. We also analyzed the genotypic RT mutations associated with ADV resistance in all patients from baseline to week 48; a single patient in ETV+TDF group had such a mutation at week 24, but this mutation was no longer present at weeks 36 and 48, and this patient achieved virologic response following ETV+TDF treatment.

**Biochemical and serologic responses**

Five patients in ETV+TDF group and 11 patients in LAM/LdT+ADV group had elevated ALT levels (\(\geq 40 \text{IU/mL}\)) at baseline. After 48 weeks, the ALT level normalized in one patient (20\%) in ETV+TDF group and in two patients (18.2\%) in LAM/LdT+ADV group (\(P = 1.000, \text{Kaplan-Meier method}\)).

Forty patients in ETV+TDF group (88.9\%) and 44 patients (95.6\%) in LAM/LdT+ADV group were HBeAg-positive at baseline. Two patients (5\%) in ETV+TDF group approached HBeAg seroconversion, and one patient (2.3\%) in LAM/LdT+ADV group achieved HBeAg seroconversion (\(P = 0.606\)).

**Table 5. Genotypic resistance to ETV (top) and ADV (bottom) from baseline to week 48 in the two study groups**

<table>
<thead>
<tr>
<th>Mutation and date</th>
<th>LAM/LdT+ADV</th>
<th>ETV+TDF</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance mutation to ETV at baseline</td>
<td>4 (8.7)</td>
<td>6 (13.3)</td>
<td>0.522</td>
</tr>
<tr>
<td>Retention of baseline mutations at week 12</td>
<td>3 (6.5)</td>
<td>2 (4.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 12</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Retention of baseline mutations at week 24</td>
<td>3 (6.5)</td>
<td>2 (4.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 24</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Retention of baseline mutations at week 36</td>
<td>3 (6.5)</td>
<td>1 (2.2)</td>
<td>0.192</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 36</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Retention of baseline mutations at week 48</td>
<td>3 (6.5)</td>
<td>1 (2.2)</td>
<td>0.106</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 48</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Resistance mutation to ADV at baseline</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Retention of baseline mutations at week 24</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 24</td>
<td>0 (0.0)</td>
<td>1 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Retention of baseline mutations at week 48</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Additional emergence of mutations at week 48</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%).

ETV, entecavir; ADV, adefovir; LAM, lamivudine; LdT, telbivudine; TDF, tenofovir.
Safety

There were 35 adverse events in 23 patients (11 in LAM/LdT+ADV group and 12 in ETV+TDF group) during the 48-week study period, and three of these events were related to the study drug (one patient with chest pain in LAM/LdT+ADV group, two patients with nausea and dyspepsia in ETV+TDF group, P=0.762). Two patients in ETV+TDF group (one with unstable angina, one with headache) and two patients in LAM/LdT+ADV group (both with HCC) discontinued therapy due to severe adverse events. However, none of these severe adverse events were related to the study drugs, and these patients improved after receiving medical treatment. None of the patients experienced elevated serum creatinine (≥0.5 mg/dL), decreased serum phosphorus (≤3 mg/dL), or serum ALT flare (>10-fold above the ULN) during the study period.

DISCUSSION

The results of this trial of patients with LAM-resistant HBV who showed suboptimal response to LAM/LdT+ADV combination therapy clearly showed that treatment with ETV+TDF provided significantly more suppression of HBV than did treatment with LAM/LdT+ADV. Moreover, continuation of LAM/LdT+ADV treatment provided little antiviral benefit, and also increased the emergence of additional RT mutations that confer resistance to ADV.

Previous studies have recommended LAM/LdT+ADV combination therapy as a treatment option for patients with LAM-resistant HBV infections. This combination therapy can prevent additional development of ADV-resistant mutations. However, as the use of continued LAM has no effect on virologic response in patients with LAM-resistant HBV, the combination of LAM/LdT+ADV provides no increased antiviral efficacy relative to ADV monotherapy. Furthermore, ADV has modest potency in suppressing HBV DNA replication, and a substantial proportion of patients have shown inadequate or suboptimal virologic responses during treatment with LAM/LdT+ADV.

Following the introduction of ETV and TDF, numerous studies have assessed the antiviral efficacy of ETV alone, ETV+ADV, TDF alone, and TDF+ETV in patients with antiviral drug-resistant HBV. ETV monotherapy is not optimal for the treatment of LAM-refractory HBV, as it leads to a lower virologic response rate in patients with LAM-resistant HBV than in LAM-naïve patients, and LAM-resistant HBV has partial resistance to ETV. In addition, genotypic resistance to ETV frequently emerges during long-term treatment of patients with LAM resistance. Prior studies showed that ETV+ADV led to better antiviral efficacy than did LAM+ADV. Furthermore, TDF-containing regimens had better antiviral efficacy compared to ETV+ADV. Recent studies showed that TDF monotherapy provided a virologic response comparable to that of TDF+ETV combination therapy, even in patients with ADV-resistant HBV and multiple-drug failures. Since none of the patients in this study was treated with TDF monotherapy, efficacy and safety could not be compared between patients who were treated with TDF and those treated with TDF+ETV. The efficacy of TDF was shown to be non-inferior to that of TDF+ETV, with TDF monotherapy associated with lower costs and lower risks of adverse events compared to TDF+ETV. Nevertheless, antiviral resistance can emerge despite resistance to TDF having a very high barrier. In this study, baseline ETV resistance remained even after 48 weeks of TDF+ETV combination therapy. Since patients with CHB require long-term antiviral therapy, resistance to highly potent antiviral agents could develop if HBV quasispecies perpetually evolve and acquire drug-resistant mutations under pressure from these antiviral agents. At that point, long-term TDF+ETV combination therapy might be a better option than long-term TDF monotherapy. To our knowledge, this is the first prospective study of patients who had LAM-resistant HBV and suboptimal responses to LAM/LdT+ADV to directly compare the efficacy of switching to ETV+TDF and continuing LAM/LdT+ADV.

This study found that the DNA suppression rate at week 48 with ETV+TDF treatment was 93.33%. This rate was higher than that observed when LAM-resistant patients were treated with ETV+TDF (84.8%), and much higher than that observed in patients infected with HBV variants that were resistant to LAM and ETV in a real-world study (51.8%). These previous studies, however, were retrospective in design. Therefore, this study is the first randomized prospective trial of South Korean patients who had LAM-resistant HBV infections and suboptimal responses to LAM/LdT+ADV. The reason for the higher virologic response observed in the present study is probably that we excluded patients with ADV resistance when enrolling the patients, as one of the treatment arms was LAM+ADV. It has been shown that the response to LAM+ADV is greatly reduced in patients with high viral loads and mutations, causing resistance to both drugs (e.g., rtA181V/T with or without rtN236T) at the initiation of treatment. However, 57.1% of the enrolled patients changed antiviral agents more than...
three times before our LAM/LdT+ADV regimen; 29.7% received ETV treatment and 8.7% had mutations associated with ETV resistance and LAM resistance. Therefore, the efficacy of TDF+ETV treatment in our patients could be considered excellent compared to previous studies. The excellent virologic response to 48 weeks of TDF+ETV combination therapy in this study suggests that combination therapy could be a better option for patients with suboptimal response to LAM/LdT+ADV for the prevention of further resistance. More specifically, 48 weeks of TDF+ETV combination therapy was highly effective in rapidly reducing the serum HBV DNA level to below the detection limit of real-time PCR, and thereby preventing the development of resistance.

Importantly, we found that the continuation of LAM/LdT+ADV treatment in patients with suboptimal responses provided little antiviral benefits to patients who had LAM-resistant HBV, and also promoted the emergence of multidrug-resistant strains of HBV. In fact, 91.3% of patients who continued on LAM/LdT+ADV experienced virologic nonresponse at week 24, and 86.9% did not experience virologic response at week 48. Furthermore, three-quarters of the patients in LAM/LdT+ADV group who had baseline ETV resistance did not achieve virologic response, and 4.7% (2/42) of patients also developed additional RT mutations responsible for ETV resistance while receiving LAM/LdT+ADV. However, with the exception of one patient with baseline ETV resistance mutation, all of the patients who were treated for 48 weeks with ETV+TDF combination treatment showed complete virologic suppression. These findings suggest that ETV+TDF combination treatment can provide virologic suppression, even if there are mutations other than those that cause LAM resistance. A previous study reported that 15% (5/33) of HBV patients without mutations that were associated with ADV resistance at baseline developed ADV resistance mutations at week 52 during continuous LAM/LdT+ADV treatment.25 We found no additional ADV resistance in LAM/LdT+ADV group, probably because we had excluded patients with ADV resistance at baseline.

Our patients generally exhibited good tolerance to each drug combination during the 48-week treatment period. No patient required dose reduction or discontinuation due to a drug-related adverse event. There were also no significant changes in serum creatinine and serum phosphorus concentrations.

This study had some limitations. First, the study duration was relatively short. Although ETV+TDF combination treatment showed excellent virologic suppression in patients with LAM-resistant HBV who had suboptimal responses to LAM/LdT+ADV, 7% (3/45) of the patients who received ETV+TDF still showed incomplete virologic suppression at week 48. Since ETV resistance can develop several years later in patients with LAM resistance, the duration of this study (48 weeks) was insufficient to examine this effect. Second, this study had an open label design, with no placebo and no blinding. Although our endpoints were objective (virologic response and biochemical response) and were determined by laboratory tests, more objective measurements of adverse events could be achieved with blinding. Finally, a recent trial showed that TDF monotherapy was comparable to ETV+TDF combination therapy in patients infected with HBV variants that were resistant to ADV and ETV.33,34 Therefore, it might be more informative to compare TDF monotherapy with ETV+TDF.

In summary, this study had three major conclusions. First, ETV+TDF led to a significantly higher rate of virologic response compared to LAM/LdT+ADV in patients with LAM-resistant HBV who had suboptimal responses to LAM/LdT+ADV regardless of the HBV genotypic resistance profile. Second, the continuation of LAM/LdT+ADV provided very little antiviral benefit, and increased the risk of RT mutations that confer drug resistance. Third, the two treatment regimens had similar safety profiles. Therefore, we conclude that promptly switching to a more potent antiviral regimen should be considered for patients who have LAM-resistant HBV infections and suboptimal responses to LAM/LdT+ADV therapy, and that ETV+TDF combination treatment is a viable option.

Authors’ contribution
Study design was by JH and SHA, data analysis and interpretation was by HYW, SHA and JH. Study write-up was by HYW. Enrollment, management of patients and data collection was by JYP, SHA, SHB, CWK, JYJ, WYT, DJK, IHK, JH. All authors had access to all data in the study and revised the article. JH, SHA and HYW made the final decision to submit the study for publication.

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Conflicts of Interest
The authors have no conflicts to disclose.
REFERENCES


Low-level viremia and cirrhotic complications in patients with chronic hepatitis B according to adherence to entecavir

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Graphical Abstract

Abstract

Adherence to Entecavir ≥ 90%

Hepatocellular Carcinoma
Cirrhotic Complications

Abbreviations:
anti-HBe, antibodies to hepatitis B e antigen; CHB, chronic hepatitis B; DM, diabetes mellitus; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; LLV, low-level viremia; MVR, maintained virologic response; NA, nucleos(t)ide analog; RFMP, restriction fragment mass polymorphism; TDF, tenofovir disoproxil fumarate; VBT, virologic breakthrough; VR, virologic response

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INTRODUCTION

Effective antiviral nucleos(t)ide analog (NA) treatment for chronic hepatitis B (CHB) using potent drugs with a high genetic barrier, such as entecavir (ETV) or tenofovir disoproxil fumarate (TDF), has been shown to regress hepatic fibrosis, prevent liver-related complications, and improve patient survival. However, the risk of hepatic complications, particularly the development of hepatocellular carcinoma (HCC), in CHB was not fully eliminated, even with potent agents. Low-level viremia (LLV) has been suggested as a possible cause of HCC in patients receiving NA treatment. According to Kim et al., LLV, defined as a lack of maintained virologic response (MVR) during ETV monotherapy, was associated with a higher risk of HCC, especially in those with cirrhosis. However, the recently updated American Association for the Study of the Liver Disease guidelines recommend that patients with LLV who are on ETV or TDF monotherapy continue monotherapy. Furthermore, nonadherence to NA treatment may lead to treatment failure. Adherence to treatment refers to the extent to which a patient takes medication as prescribed and for the duration of treatment agreed upon by the patient and physician. In real-world clinical settings, nonadherence is common and can restrict the full benefits of antiviral treatment. Treatment nonadherence is likely to be a more significant contributor to treatment failure than antiviral resistance against anti-hepatitis B virus (HBV) agents, such as ETV, which exhibit potent viral suppression with a lower risk of drug resistance.

This study aimed to evaluate the effect of LLV on HCC development, mortality, and cirrhotic complications in patients with CHB according to their adherence to ETV (ETV) treatment.
PATIENTS AND METHODS

Patients

In this retrospective longitudinal observational study, we used the electronic medical records of patients with treatment-naïve CHB who received ETV therapy between January 1, 2007 and January 31, 2017 at Ulsan University Hospital, a tertiary referral center in South Korea. Patients were eligible for inclusion at the time of the initiation of antiviral treatment if they met the following criteria: age ≥18 years; hepatitis B surface antigen (HBsAg)-positive for >6 months or CHB defined by clinical history; no malignancy including HCC at baseline; no evidence of viral coinfections (i.e., human immunodeficiency virus, hepatitis C virus, or hepatitis D virus); no alcohol-related liver disease or autoimmune hepatitis; and treatment-naïve prior to starting ETV 0.5 mg per day (ETV has been available in South Korea since January 1, 2007). The exclusion criteria included the following: follow-up duration <1 year; evidence of decompensated cirrhosis as indicated by the presence (or history) of ascites, esophageal, or gastric variceal bleeding, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, liver transplantation, or a Child-Pugh score ≥7; serum creatinine level >1.5 mg/dL at baseline; HBV DNA <2,000 IU/L at baseline; or death within 6 months of treatment initiation. Patients who developed HCC within the first year of enrollment were also excluded to minimize the inclusion of pre-existing unidentified HCC and the misattribution of treatment effect. We also excluded patients with renal disease who needed dose adjustments that could affect the adherence rate. Finally, 894 patients were enrolled. Information about baseline patient characteristics and clinical outcomes were obtained from complete inpatient and outpatient medical records. This cohort was originally investigated by Shin et al., and clinical progress was further updated through August 31, 2018 for the current study. This study was approved by the Institutional Review Board of Ulsan University Hospital (#IRB No. 06-2017-26). The requirement for informed consent was waived, as patient records and information were de-identified prior to analysis.

Serum assay

Whole blood count, biochemical and HBV virologic markers, and serum HBV DNA levels were assessed for all patients every 3–6 months during ETV therapy. Serum HBV DNA levels were quantified using the COBAS® TaqMan HBV test (Roche, Branchburg, NJ, USA), which has a lower detection limit of 12 IU/mL (60 copies/mL). Levels of HBsAg, hepatitis B e-antigen (HBeAg), and antibodies to hepatitis B e-antigen (anti-HBe) were examined using an enzyme immunoassay. The virologic outcomes included virologic response (VR), virologic breakthrough (VBT), and MVR or LLV during the on-treatment follow-up period. VR was defined as undetectable serum HBV DNA by polymerase chain reaction assay (<12 IU/mL) for two consecutive measurements during ETV treatment. VBT was defined as an increase of >1 log_{10} IU/mL in serum HBV DNA level from nadir for two consecutive measurements or on the last available measurement. Based on the serum HBV DNA levels during the follow-up period, patients were categorized as either MVR or LLV at the last follow-up, as mentioned in a previous study by Kim et al. MVR was defined as having serum HBV DNA persistently undetectable throughout the follow-up period after achieving a VR. The remaining patients showed either persistent or intermittent episodes of detectable serum HBV DNA <2,000 IU/mL during follow-up, which was defined as LLV. Restriction fragment mass polymorphism (RFMP; Genematrix®, Youngin, Korea) was used to identify ETV-resistant mutations in the HBV polymerase gene following VBT occurrence during the treatment period. The RFMP assay can detect 100 copies of HBV genome/mL. For this study, we have further investigated and updated the history of diabetes mellitus (DM) and hypertension. DM was defined as a self-reported history of diabetes, use of anti-diabetic medication, and/or those with a fasting plasma glucose ≥126 mg/dL. Hypertension was defined as blood pressure ≥140/90 mmHg or a self-reported history of hypertension and/or use of anti-hypertensive medication.

Follow-up assessments

During the follow-up period, all patients underwent periodic surveillance with ultrasonography and laboratory workups, including α-fetoprotein and protein-induced vitamin K absence or antagonist-II, every 6 months to screen for HCC. Liver cirrhosis was clinically defined based on repeated liver imaging studies (nodular liver surface or caudate lobe hypertrophy) with thrombocytopenia (<150×1,000/mm^3) or splenomegaly (by imaging), and/or by the presence of varices (by upper endoscopy or imaging studies). HCC diagnosis was confirmed using radiology (dynamic computed tomography and/or magnetic resonance imaging), as recommended by international guidelines. The clinical outcomes were the cumulative incidence of liver-related death or transplantation, HCC, and hepatic decompensation (ascites, variceal bleeding,
spontaneous bacterial peritonitis, hepatic encephalopathy, and hepatorenal syndrome). Liver-related death was defined as death related to cirrhotic complications and HCC. To avoid statistical repetition, the earliest cirrhotic complication was selected if a given patient experienced different types of cirrhotic complications at different time points.

Medication adherence

Medical and pharmacy refill records were reviewed to assess medication adherence by prescription records and the actual amount of medication taken by the patient. Adherence rate was expressed as the percentage of days the patient had medications in his/her possession during the period in which he/she was undergoing therapy. This proportion was calculated as the sum of days on which medication was supplied (obtained over a series of intervals) divided by the total treatment duration (days), which was derived from the dates of the first and last prescriptions dispensed. The medication from the final prescription refill was not included, as its consumption was unknown. Good adherence to medication was defined as a cumulative adherence ≥90% per study period among patients who were prescribed ETV in a given period. The cutoff of 90% was previously used in a similar study.20,24

Statistical analyses

Continuous variables were compared using the Student’s t-test or Mann-Whitney U-test, and categorical variables were compared using the chi-square test or Fisher’s exact test. Serum HBV DNA (IU/mL) levels were logarithmically transformed for analyses. All statistical tests were considered significant with two-sided P-values of <0.05. Cumulative probabilities of the clinical outcomes were calculated using the Kaplan-Meier method. Univariate and multivariate analyses for factors predictive of the development of HCC were performed using a Cox proportional-hazard model. The results of the model were presented as a hazard ratio (HR) with a 95% confidence interval (CI). Variables were not included in the multivariate model if P-values in the univariate analysis were >0.2. Statistical analyses were performed using the statistical package SPSS for Windows (version 24.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics and virologic outcomes in the entire cohort

Overall, 1,955 patients with NA-naïve CHB were consecutively treated with ETV during the study period from January 1, 2007 to August 31, 2018. Among these patients, 894 were considered eligible for analysis (Supplementary Fig. 1). The demographic and clinical characteristics of all patients included in this study are summarized in Table 1. VR was observed in 812 patients (90.8%). The median time to VR was 0.8 years (range, 0.1–6.6). The cumu-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=894)</th>
<th>MVR (n=654)</th>
<th>LLV (n=240)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>52.0±11.4</td>
<td>52.4±11.2</td>
<td>50.8±11.7</td>
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<td>Male sex</td>
<td>597 (66.8)</td>
<td>418 (63.9)</td>
<td>179 (75.6)</td>
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<td>HBeAg positivity</td>
<td>537 (60.1)</td>
<td>362 (55.4)</td>
<td>175 (72.9)</td>
<td>&lt;0.001</td>
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<tr>
<td>Cirrhosis</td>
<td>440 (49.2)</td>
<td>330 (50.5)</td>
<td>110 (45.8)</td>
<td>0.220</td>
</tr>
<tr>
<td>HBV DNA (log_{10} IU/mL)</td>
<td>6.4±1.4</td>
<td>6.3±1.4</td>
<td>6.6±1.5</td>
<td>0.011</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>143.7±173.1</td>
<td>143.6±177.6</td>
<td>141.9±158.5</td>
<td>0.890</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>4.2±0.4</td>
<td>4.2±0.4</td>
<td>4.1±0.5</td>
<td>0.093</td>
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<td>Prothrombin time (INR)</td>
<td>1.0±0.2</td>
<td>1.0±0.2</td>
<td>1.0±0.3</td>
<td>0.377</td>
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<td>Platelet (×1,000/mm³)</td>
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<td>169.9±63.0</td>
<td>169.1±63.0</td>
<td>0.872</td>
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<td>Diabetes mellitus</td>
<td>150 (16.8)</td>
<td>105 (16.1)</td>
<td>45 (18.8)</td>
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</tr>
<tr>
<td>Hypertension</td>
<td>98 (11.0)</td>
<td>74 (11.3)</td>
<td>24 (10.0)</td>
<td>0.630</td>
</tr>
</tbody>
</table>

Table 1. Baseline characteristics in all patients

Values are presented as mean±standard deviation or number (%).
MVR, maintained viral response; LLV, low-level viremia; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; INR, international normalized ratio.
Relative VR incidence rates were 66.3%, 85.2%, and 95.2% at 1, 3, and 5 years, respectively. Among the patients, MVR was achieved in 654 (73.2%), and LLV remained in 240 (26.8%). During follow-up, 125 patients (14.0%) experienced VBT (good adherence group, n=30; poor adherence group, n=95), with lower rates in good adherence group than in poor adherence group (4.9% vs. 34.3%, P<0.001). The cumulative probability of VBT was 0.6%, 6.7%, 10.8%, and 12.4% at years 1, 3, 5, and 7, respectively. Good adherence to ETV medication was observed in 617 patients (69.0%), and 277 patients (31.0%) had poor adherence. Among the patients with good adherence, MVR was achieved in 509 (82.5%), and LLV remained in 108 (17.5%) (Table 2). Kaplan-Meier survival analysis demonstrated a significantly higher risk of LLV in poor adherence group than in good adherence group (log-rank P<0.001; Fig. 1). There were a total of 42 cases of HBV DNA ≥2,000 IU/mL in 125 VBT patients. Among them, 17 patients (including 11 patients with ETV-resistant mutations) were eventually replaced by TDF, at which point the follow-up for the study was censored. For the remaining 25 patients, at HBV DNA ≥2,000 IU/mL, the compliance was investigated to encourage ETV medication. As a result, within 3 months (next follow-up test) for 23 and within 6 months for 2 patients, HBV DNA was reduced to below 2,000 IU/mL, in which case ETV treatment was continued. The 25 patients who continued to take ETV even though HBV DNA was ≥2,000 IU/mL did not show ≥2,000 IU/mL again in their subsequent observations, and all of them were considered LLV in this analysis. Among the patients with LLV, 80 underwent mutation tests and 22 developed ETV-resistant mutations (L180M/ M204V/S202G [n=13]; L180M/M204V/T184LSM [n=5]; L180M/ M204V/M250L [n=4]). The cumulative probability of acquiring a genotypic ETV mutation was 0%, 1.5%, 2.7%, and 3.5% at years 1, 3, 5, and 7, respectively. Regarding LLV, 56/240 patients received TDF, and for statistical analysis, those patients were censored after they switched from ETV to TDF. Of the 56 TDF replacement patients, 45 were caused by VBT and 11 were non-VR cases (ETV-resistant mutations were existed in all non-VR cases).

![Figure 1. Cumulative incidence of low-level viremia according to good adherence vs. poor adherence.](image)

**Table 2.** Baseline characteristics in good adherence group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=617)</th>
<th>MVR (n=509)</th>
<th>LLV (n=108)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.6±11.1</td>
<td>52.9±11.0</td>
<td>51.1±11.7</td>
<td>0.156</td>
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<tr>
<td>Male sex</td>
<td>400 (64.8)</td>
<td>313 (61.5)</td>
<td>87 (80.6)</td>
<td>&lt;0.001</td>
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<tr>
<td>HBeAg positivity</td>
<td>362 (58.7)</td>
<td>281 (55.2)</td>
<td>81 (75.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>297 (48.1)</td>
<td>257 (50.5)</td>
<td>40 (37.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>HBV DNA (log$_10$ IU/mL)</td>
<td>6.4±1.4</td>
<td>6.3±1.4</td>
<td>6.5±1.5</td>
<td>0.175</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>138.3±171.8</td>
<td>141.6±176.8</td>
<td>122.6±145.2</td>
<td>0.238</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.2±0.4</td>
<td>4.2±0.4</td>
<td>4.1±0.3</td>
<td>0.246</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>1.0±0.2</td>
<td>1.0±0.2</td>
<td>1.0±0.1</td>
<td>0.624</td>
</tr>
<tr>
<td>Platelet (×1,000/mm$^3$)</td>
<td>171.5±62.9</td>
<td>170.2±62.2</td>
<td>177.6±65.6</td>
<td>0.288</td>
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<tr>
<td>Diabetes mellitus</td>
<td>100 (16.2)</td>
<td>77 (15.1)</td>
<td>23 (21.3)</td>
<td>0.116</td>
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<tr>
<td>Hypertension</td>
<td>64 (10.4)</td>
<td>55 (10.8)</td>
<td>9 (8.3)</td>
<td>0.602</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%).
MVR, maintained viral response; LLV, low-level viremia; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; INR, international normalized ratio.
Clinical outcomes of the entire cohort

The clinical outcomes of the entire cohort, according to MVR vs. LLV, are shown in Figure 2. During the follow-up period of up to 132 months, HCC developed in 91/894 patients (10.2%): 58/654 (8.7%) in MVR group and 33/240 (13.8%) in LLV group. The cumulative incidence of HCC was significantly lower in MVR group than in LLV group: 5- and 10-year cumulative incidence rates of HCC in MVR vs. LLV were 7.8 vs. 11.5% and 12.8 vs. 20.1%, respectively (log-rank \(P=0.017\)). Liver-related death or transplantation occurred in 22/894 patients (2.5%) (14/654 [2.1%] in MVR group and 8/240 [3.3%] in LLV group), and hepatic decompensation occurred in 44/894 patients (4.9%) (27/654 [4.1%] in MVR group and 17/240 [7.1%] in LLV group). However, in terms of other clinical outcomes, no statistically significant difference was found between MVR and LLV groups (log-rank \(P=0.245\) for liver-related death or transplantation; log-rank \(P=0.050\) for hepatic decompensation).

Clinical outcomes of good adherence group

Clinical outcomes according to MVR vs. LLV in good adherence group are shown in Figure 3. Overall, during the follow-up period of up to 132 months, HCC developed in 43/617 patients (7.0%):
36/509 (7.1%) in MVR group and 7/108 (6.5%) in LLV group. Liver-related death or transplantation occurred in 4/617 patients (0.6%) (4/509 [7.9%] in MVR group and 0/108 [0.0%] in LLV group), and hepatic decompensation occurred in 12/617 (1.9%) patients (12/509 [2.4%] in MVR group and 0/108 [0.0%] in LLV group). The cumulative incidence of liver-related death or transplantation, HCC, and hepatic decompensation showed no significant differences between MVR and LLV groups (log-rank $P=0.359$ for liver-related death or transplantation; log-rank $P=0.937$ for HCC; log-rank $P=0.118$ for hepatic decompensation). The development of HCC also showed no difference between MVR and LLV groups in the subgroup analysis regarding cirrhotic subcohort (log-rank $P=0.536$) (Supplementary Fig. 2).

**Risk factors for HCC**

Multivariate analyses were performed to compare the effect of LLV to other well-known risk factors for the development of HCC in the entire cohort and good adherence group (Table 3). Multivariate analysis showed that among the entire cohort, in addition to the traditional risk factors for HCC such as old age, male sex, and cirrhosis, MVR presented a statistically significant association with the development of HCC with a HR of 0.61 (95% CI, 0.39–0.96; $P=0.031$). However, in the analyses of good adherence...
group, LLV showed no independent association with HCC ($P=0.967$), whereas the traditional risk factors for HCC, such as age, male sex, and cirrhosis, presented strong associations with the development of HCC in multivariate analysis.

**DISCUSSION**

According to multivariate analysis, LLV during treatment was not a predictive factor for HCC and cirrhotic complications in patients with CHB and good adherence to ETV treatment. With the development of antiviral agents, a dramatic change has occurred in the treatment and prognosis of CHB. In particular, potent antiviral agents with high genetic barriers enabled continued use of medication and showed long-term results in reduced mortality and the progression of cirrhosis or HCC. However, even with the prolonged use of antiviral agents, complications of CHB, particularly HCC, are not fully inhibited. A multicenter study which investigated the incidence of HCC in 1,666 Caucasian patients with CHB receiving ETV or TDF showed that the cumulative probability of HCC was 3.4% at year 3 and 8.7% at year 5, with an annual incidence rate of 1.37% per year. The annual incidence of HCC in Caucasian patients with compensated HBV-related cirrhosis was about 2.2%, and the 5-year cumulative incidence was about 10%. Previous studies of Korean patients showed that even with optimal VR under NA therapy, the annual incidence of HCC was 0.15–0.80% per year in non-cirrhotic patients, and 0.95–3.55% per year in compensated-cirrhotic patients.

LLV has been suggested as a possible cause of HCC in patients receiving NA treatment. A previous study showed that LLV, defined as a lack of MVR, significantly increases the incidence of HCC in patients with CHB who are receiving ETV. Sub-optimally suppressed viral replication in the liver can cause continuous inflammation, and the proliferation of inserted HBV oncogenes is also considered an important mechanism of liver cancer. In a previous study, patients with LLV in whom a continuous viral response was not achieved had an increased cumulative incidence of HCC, which was more noticeable in patients with cirrhosis. This was also evident in our study; subgroup analysis of the cirrhotic subcohort showed that the cumulative incidence of HCC and hepatic decompensation were significantly lower in MVR group than in LLV group (log-rank $P=0.001$ and log-rank $P=0.011$, respectively) (Supplementary Fig. 3).

Meanwhile, in another study, we presented poor treatment adherence as a significant risk factor for mortality, HCC, and hepatic decompensation. Data on adherence to NA treatment for CHB are limited. In a previous study, a pharmacy claims database with 11,100 patients receiving NAs for CHB was used to investigate the rates of patient adherence in a real-world setting. The overall adherence rates were found to be 87.8%, with 55.3% of patients having ≥90% adherence. Other studies have reported mean NA treatment adherence rates ranging from 81% to 99%.

| Table 3. Multivariate analysis of potential risk factors for hepatocellular carcinoma in the entire cohort and good adherence subcohort |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Characteristic                  | Entire cohort (n=894)            | Good adherence subcohort (n=617) |
|                                 | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Age                             | 1.06 (1.03–1.09) | <0.001 | 1.05 (1.01–1.10) | 0.024 |
| Male sex                        | 4.77 (2.57–8.87) | <0.001 | 9.00 (3.05–26.60) | <0.001 |
| HBeAg positivity                | 0.86 (0.53–1.40) | 0.536 | 0.84 (0.41–1.71) | 0.624 |
| Cirrhosis                       | 6.10 (2.79–13.32) | <0.001 | 5.18 (1.82–14.75) | 0.002 |
| MVR                             | 0.61 (0.39–0.96) | 0.031 | 1.02 (0.44–2.38) | 0.967 |
| HBV DNA (log₁₀ IU/mL)           | 0.91 (0.76–1.09) | 0.312 | 0.83 (0.63–1.09) | 0.185 |
| ALT (IU/mL)                     | 1.00 (1.00–1.00) | 0.184 | 1.00 (1.00–1.00) | 0.596 |
| Albumin (g/dL)                  | 0.65 (0.39–1.08) | 0.093 | 0.46 (0.19–1.10) | 0.079 |
| Prothrombin time (INR)          | 1.86 (0.72–4.80) | 0.200 | 0.38 (0.02–6.21) | 0.495 |
| Platelet (x1,000/mm³)           | 1.00 (0.99–1.00) | 0.613 | 1.00 (0.99–1.01) | 0.767 |
| Diabetes mellitus               | 1.37 (0.86–2.20) | 0.190 | 1.73 (0.87–3.42) | 0.118 |
| Hypertension                    | 2.09 (1.27–3.45) | 0.004 | 2.82 (1.32–6.02) | 0.007 |

HR, hazard ratio; CI, confidence interval; HBeAg, hepatitis B e antigen; MVR, maintained viral response; HBV, hepatitis B virus; ALT, alanine aminotransferase; INR, international normalized ratio.
was consistent with the mean adherence rate of 89.1% observed in the current study.\textsuperscript{20} Despite the significance of viral suppression to prevent cirrhotic complications and mortality, evidence on the association between adherence to ETV treatment and hepatic complications still remain limited. In our previous study, we evaluated the association of adherence to ETV treatment on hepatic complications in patients with CHB.\textsuperscript{20} Multivariate Cox proportional regression analyses demonstrated that adherence to ETV treatment was a powerful factor for HCC development, cirrhotic complications, and liver-related or all-cause mortality. In the same study, continuous viral response and MVR increased, whereas VBT was significantly low in patients with good adherence, indicating the correlation between adherence and viral response. These results suggest that poor adherence to antiviral agents is closely linked to LLV. In our current study, when we assessed the correlation between LLV and adherence, LLV was significantly associated with the development of HCC with a HR of 0.61 (95% CI, 0.39–0.96; \( P =0.031 \)), in addition to the other risk factors: old age, male sex, and cirrhosis. On the other hand, among patients with MVR (n=654), HCC developed in 36/509 patients (7.1%) in good adherence group and 22/145 (15.2%) in poor adherence group during the follow-up period of up to 132 months. Kaplan-Meier survival analysis demonstrated a significantly higher risk of HCC in poor adherence group compared to good adherence group (log-rank \( P =0.001 \)). Liver-related death or transplantation occurred in 4/509 (0.8%) in good adherence group and 10/145 (6.9%) in poor adherence group. Hepatic decompensation occurred in 12/509 (2.4%) in good adherence group and 15/145 (10.3%) in poor adherence group. Kaplan-Meier survival analysis demonstrated a significantly higher risk of liver-related death or transplantation, as well as hepatic decompensation, in poor adherence group compared to good adherence group (log-rank \( P =0.001 \)).

In this study, adherence was shown to be a more significant factor than LLV for predicting the occurrence of cirrhotic complications. However, MVR vs. LLV and good vs. poor adherence are not necessarily different concepts, and both are indicators that reflect the intra-hepatic viral replication of HBV. In the statistics of our study, the result that LLV does not appear to be an independent risk factor for HCC occurrence does not infer that LLV is not related to HCC occurrence, but is rather a confounding factor closely related to adherence. The ideal viral suppression is a key mechanism to prevent cirrhotic complications in patients with CHB, such as HCC. In general, LLV is based on a serologic test for HBV DNA conducted at 3-to-6-month intervals, with the limitation that viral replication status in the middle of testing is unknown. In contrast, nonadherence refers to a period of inadequate medication during follow-up, suggesting the possibility of viral proliferation during the period of nonadherence. In other words, as sustained antiviral therapy is important for HBV inhibition, theoretically, adherence can be considered as a continuous variable that more accurately reflects intra-hepatic viral loads compared to LLV with periodic serologic testing.

A previous study of Korean patients with optimal VR during NA therapy showed that the cumulative HCC incidence was 8.1% at 3 years and 17.4% at 5 years with baseline liver cirrhosis, and 4.7% at 3 years and 7.2% at 5 years without baseline liver cirrhosis.\textsuperscript{9} In our study, the 5- and 10-year cumulative incidence rates of HCC...
in good adherence vs. poor adherence were 5.8 vs. 15.9% and 10.3 vs. 26.0%, respectively, in the entire cohort (Supplementary Fig. 4). In terms of cirrhotic subcohort, the 5- and 10-year cumulative incidence rates of HCC in good adherence vs. poor adherence were 10.0 vs. 28.9% and 17.1 vs. 44.1%, respectively (Supplementary Fig. 5). These results suggest that the long-term preventive effects of ETV treatment may be significantly attenuated in patients with poor adherence. This again presents the importance of continuous and regular medication in patients with CHB.

The strengths of this study were its large sample size and long-term follow-up, which together allowed for increased statistical power and greater reliability of data. We also sought to analyze from multiple perspectives to clarify the relationship between adherence, LLV, and cirrhotic complications. Nonetheless, this study had some limitations. First, the retrospective nature of certain clinical information might be biased due to incomplete data collection. The common reasons affecting patient adherence, such as lack of recognition, forgetfulness, overriding priorities, and emotional or cultural factors, may be expected to occur in patients prescribed NA therapy. However, due to the retrospective nature of this study, the causes could not be identified in approximately 30% of the patients. Second, individuals who developed LLV had different durations of LLV during the follow-up period, which may have had different degrees of impact on clinical outcomes. Furthermore, defining LLV or MVR is subject to the frequency and intervals of HBV DNA testing and may not be the same for all patients, which may result in classification bias. Third, since the number of patients with cirrhosis and LLV despite good adherence to treatment was very low (n=40, only six cases of HCC), it was too statistically low-powered to draw a definite conclusion regarding the effect of LLV on clinical outcomes among the cirrhotic subgroup. Fourth, since our database did not include other NAs (lamivudine, adefovir dipivoxil, telbivudine, and TDF) besides ETV, the application of these results to other NAs should be considered with caution. Finally, although pharmacy refill records are objective measures and are routinely collected, they do not measure whether the participants actually took their medications. The rates of nonadherence may have been overestimated if the dispensed medications were not used. In addition, identifying and understanding the factors influencing nonadherence to medication is necessary to identify appropriate interventions.

In conclusion, no significant difference was found between MVR and LLV groups in terms of the incidence of liver-related death or transplantation, HCC, and hepatic decompensation in patients with good adherence. For patients with suboptimal virological response, it is helpful to check their adherence before considering to continue, switch, or add another drug. For patients with good adherence who experience LLV during ETV treatment, it may be unnecessary to adjust their antiviral agent immediately, and careful observation would be possible.

Authors’ contribution

Study coordination and design, data collection, data analysis, statistical analysis, writing and revision of the manuscript, and approval of the final version: SBL, BRP, and EJP. Study coordination and design, data analysis, critical review of the manuscript, and approval of the final version: NHP and JWS. Data supply, critical review of the manuscript, and approval of the final version: JJ, JHP, SWJ, IDJ, and SJB. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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


Dear Editor,

We read with great interest the article entitled “Low-level viremia and cirrhotic complications in patients with chronic hepatitis B according to adherence to entecavir” by Lee et al. In that study, the authors reported that low-level viremia (LLV) was not a predictive factor for the development of hepatocellular carcinoma (HCC) and cirrhotic complications in patients with good adherence to entecavir (ETV) therapy. This study used the same study population as a previous report of an association between LLV and a higher risk of HCC development in Korean patients with cirrhosis. Several issues should be kept in mind when interpreting the results of the study.

The first issue of concern is the accuracy of the criteria defining good or poor adherence to ETV therapy. The adherence rate was calculated as the sum of the days on which the medication was supplied divided by the total treatment duration, defined based on the dates of the first and last dispensed prescriptions. However, it is difficult to distinguish between good and poor adherence retrospectively. Although pharmacy refill records are objective, they of course contain no information on whether the patients actually took their medications. Therefore, daily monitoring of the patient such as by a diary or phone call is necessary, otherwise, recall bias can occur. Considering that patients who visit a tertiary hospital usually have good compliance, one third of the poor adherence in the study cohort might have been overestimated. This limitation can be resolved by a prospective study or a determination of the factors influencing adherence to ETV, to identify appropriate interventions.

Second, the study by Shin et al. showed that poor adherence to ETV increased the risks of mortality, HCC and cirrhotic complications, particularly among patients with cirrhosis. In the study by Lee et al., LLV increased the HCC risk compared to a maintained virological response (MVR), but there was no difference between LLV and MVR among patients with good adherence. Poor adherence to medication results in less viral suppression, which in patients with chronic hepatitis B (CHB) will affect long-term prognosis, including a higher risk of HCC or cirrhotic complications. Which factor is more important: LLV or the presence of cirrhosis? The American Association for the Study of Liver Diseases guidelines suggest that patients with LLV should continue monotherapy rather than take a second antiviral drug. High genetic barrier antivirals such as ETV or tenofovir failed to achieve HBV DNA undetectability after 48 weeks in 10% and 30% of hepatitis B e antigen negative and positive patients with CHB. According to Lee et al., ETV therapy should be continued in patients with good ad-
herence. However, the authors did not show differences according to the presence of cirrhosis among patients with good adherence. A detailed study of when to test for ETV resistance and when to add or change antiviral drugs is needed.

Third, the definition of MVR vs. LLV is an important issue. In the study by Lee et al., patients were categorized as MVR or LLV at the last follow-up. However, despite an update of the study period, the number of patients with good adherence was the same as in the study by Shin et al. How many HBV DNA measurements were made in each patient and how frequently? It may have been the case that the good adherence group had more frequent blood tests, including determinations of HBV-DNA levels. If the poor adherence group had longer measurement intervals than the good adherence group then, in the former, LLV may have been classified as MVR.

Lastly, Cho et al. reported that patients with a complete virological response (HBV DNA <20 IU/mL) had significantly longer overall survival compared to patients with fluctuating HBV DNA levels (20–2,000 IU/mL), regardless of antiviral treatment. In addition, among patients receiving nucleos(t)ide analogues, overall survival was significantly longer in those obtaining a complete virological response than in those obtaining a partial virological response. However, all of the cited previous studies were retrospective. A prospective study in a multicenter setting is currently in progress and should address this issue.

In conclusion, the study contributes to a better understanding of LLV as a prognostic factor in patients with CHB based on ETV adherence. Although no prospective data are currently available, the prognosis of patients who have LLV merits close attention regardless of ETV treatment, especially that of patients with cirrhosis.

Authors’ contribution
HW Lee: drafting of manuscript; BK Kim: critical revision and supervision

Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Dear Editor,

An increase in serum aspartate aminotransferase (AST) may be related to different conditions, including viral hepatitis, alcoholic liver disease, cirrhosis, cholestatic syndromes, acute myocardial infarction, or skeletal muscle trauma.¹ Chronic and isolated elevation of AST in patients with no related clinical signs or symptoms is suggestive for the presence of macro-aspartate aminotransferase (macroAST).² MacroAST is a macroenzyme that circulates in the bloodstream as a high molecular weight complex, either by self-polymerisation or by association with serum proteins such as immunoglobulins (Ig).¹ The formation of this complex, particularly with Ig, may lead to increased activity and/or reduced inactivation, clearance or excretion.¹,³ Below is a case report of a healthy woman with elevated AST levels due to the presence of macroAST.

A 45-year-old female was admitted to the gastroenterology department of Careggi Hospital in Florence, because of an isolated increase in AST levels. Family history was positive for arterial hypertension and stroke. The patient had a body mass index of 22.0, and her clinical history showed that she had an ovarian cyst 5 years ago and had taken oral contraceptives from the age of 18 to 35 years. No major findings were present at clinical examination. Abdominal ultrasound did not reveal any alterations, except for a newly formed ovarian cyst on the left side. The electrocardiogram did not show any abnormalities. Blood tests at admission showed: AST 149 U/L (reference interval, 15–37); alanine aminotransferase 19 U/L (reference interval, 12–65); creatine phosphokinase 95 U/L (reference interval, 21–215); haptoglobin 1.01 g/L (reference interval, 0.3–2); troponin I <0.015 μg/L (reference interval, 0–0.09); myoglobin 42 ng/mL (reference interval, 14–106); and creatine kinase-MB 0.7 ng/mL (reference interval, 0.5–3.6). The levels of lactate dehydrogenase and aldolase were also normal. Diagnostic testing for thyroid disease, muscle disorders, haemolysis and coeliac disease was negative. Serology of viral hepatitis B, C, A, Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus was also negative.⁴ Values of the subsequent laboratory investigations were constant and varied only slightly over time. All other haematological and biochemical parameters were normal. Presence of macroAST was hypothesised by the gastroenterologist and the clinical laboratory was asked to perform additional tests to confirm this diagnosis.

For the detection of macroAST, the polyethylene glycol (PEG) precipitation method was used by the clinical laboratory as for the evaluation of macroprolactin.⁵ Here, 200 μL of serum was added to an equal volume of PEG 6000 (Merck, Milano, Italy) 250 g/L in distilled water, vortex-mixed for 1 minute and centrifuged at 1,500 rpm for 30 minutes at 4°C, resulting in a clear supernatant with a precipitate at the bottom. The PEG solution was prepared fresh every 3 months and stored at 4°C.⁵ Simultaneously, 100 μL...
of serum was mixed with 100 μL phosphate-buffered saline (PBS). AST activities were measured both on the supernatant and on the PBS dilution with a Siemens VISTA Clinical Chemistry System (Siemens Healthcare, Milano, Italy) and were adjusted with a correction factor of two for the dilution in the preparation. AST recovery was derived as a percentage of the AST activity measured in the supernatant relative to that measured in the PBS dilution. To evaluate the effect of PEG precipitation, a serum sample of a patient previously diagnosed with hepatopathologic disease and negative for macroAST was selected and treated in the same way as the clinical case serum sample.

Table 1 shows AST activity after PEG precipitation in the patient from the case report and in the control patient. A recovery of AST activity ≤40% in cases of suspected macroAST indicates the presence of Ig-AST complexes, while this condition is very unlikely at values of AST recovery >50%. The use of PEG at defined concentrations has the effect of subtracting the solvents, and the subsequent precipitation of proteins such as Ig and the complexes formed by them, thus measuring the remaining activity in the supernatant. If macroAST is present, the activity of the enzyme after PEG precipitation is decreased compared to the control. Given this case report finding, the results are consistent with a diagnosis of macroAST and confirm the clinical suspicion. Figure 1 shows how the AST changed from 30, 10 days before diagnosis and during follow-up at 10, 30, and 120 days after diagnosis.

Recently, reports about this disease are increasing; Table 2 collects the macroAST literature previously reported, comparing similarities and differences of each case report. The presence of macroAST can be determined, as shown in Table 2, by laboratory techniques including gel filtration chromatography, ultrafiltration, immunofixation electrophoresis, Ig depletion using protein A and G, refrigerated sample storage for 3–6 days at 4°C and PEG precipitation. In gel filtration chromatography, the molecules are separated according to their size and shape. In this method, the stationary phase is made up of spheres of hydrated material, containing pores that can be crossed only by molecules with certain dimensions. In this way, molecules with dimensions that are too large will cross the column very quickly and be eluted into a smaller volume than the molecules that enter the pores instead. Ultrafiltration is based on the molecular weight of macroAST for separation. It is performed using centrifugal filter units with membranes, and AST activity is measured in the ultracentrifugate after centrifugation for 18 hours. Electrocentrifugation and gel filtration chromatography, which constitute the standard reference method, require highly specialised chromatography, are complex, relatively expensive, and time-consuming and are not available in most clinical laboratories. Electrophoresis for immunofixation with AST staining consists of the electrophoresis of serum proteins using the IgG, IgA and IgM antisera. It allows the nature of enzyme immune complexes to be clarified, but requires high specialisation skills. For the Ig depletion method using protein A and G, instead, proteins A and G are recombinant bacterial proteins used to remove Ig and Ig-AST complexes after incubation with the patient’s serum. AST is determined in the supernatant after centrifugation of the sample. The protein A and G method is straightforward and provides unambiguous results, but this method is more expensive and probably only detects AST-IgG macrocomplexes. Moreover, refrigerated sample storage for 3–6 days at 4°C determines the gradual precipitation of the enzyme-Ig complex. This method is simple and reliable, but it is performed over a long time.

Table 1. AST activity

<table>
<thead>
<tr>
<th></th>
<th>Serum AST (U/L)</th>
<th>PEG AST (U/L)*</th>
<th>PBS AST (U/L)*</th>
<th>AST % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>149</td>
<td>8</td>
<td>143</td>
<td>5.6</td>
</tr>
<tr>
<td>Control</td>
<td>338</td>
<td>262</td>
<td>270</td>
<td>97</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; PEG, polyethylene glycol; PBS, phosphate-buffered saline.

*The results are multiplied by the dilution factor 2.
<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>Sex</th>
<th>Country</th>
<th>Clinical features</th>
<th>Diagnostic analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Wijk et al.¹ (2016)</td>
<td>50</td>
<td>F</td>
<td>China</td>
<td>Hypertension, hypertriglyceridemia, pre-diabetes mellitus, HBV; occasional muscle aches in right shoulder and legs. Taking statin.</td>
<td>Ig depletion using protein A and G; PEG precipitation and ultrafiltration</td>
</tr>
<tr>
<td>Wener et al.³ (2012)</td>
<td>34</td>
<td>F</td>
<td>Poland</td>
<td>Severe abdominal and pelvic trauma, nephrolithiasis, benign ovarian cyst; pain in the right upper abdomen; no fever. Occasional intake of antacid.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Lee et al.⁴ (2011)</td>
<td>25</td>
<td>F</td>
<td>USA</td>
<td>Paternal dyslipidemia; older brother with thyroid cancer. Mild fatigue. Occasional intake of FANS.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Patteet et al.⁵ (2012)</td>
<td>27</td>
<td>F</td>
<td>Belgium</td>
<td>Migraine treated with topiramate. Tiredness.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Bustamante et al.⁶ (2016)</td>
<td>23</td>
<td>F</td>
<td>Chile</td>
<td>Paternal Gilbert’s syndrome; uncle died of multiple myeloma. Syndrome polycystic ovary. Taking oral contraceptive. No smoke, no alcohol.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Zhan et al.⁸ (2019)</td>
<td>34</td>
<td>F</td>
<td>China</td>
<td>No relevant family history. Infertile; mild cholecystitis. Taking drugs for liver protection and traditional Chinese medicine for infertility. No alcohol.</td>
<td>Refrigerated sample storage; Ig depletion using protein A and G; electrophoresis</td>
</tr>
<tr>
<td>Ono et al.⁹ (2019)</td>
<td>22</td>
<td>F</td>
<td>Japan</td>
<td>No relevant family history. Common cold. No taking medicine.</td>
<td>GFC; refrigerated sample storage; Ig depletion using protein A and G; electrophoresis</td>
</tr>
<tr>
<td>González Raya et al.¹⁶ (2019)</td>
<td>50</td>
<td>F</td>
<td>Spain</td>
<td>No relevant family history. Asymptomatic. No alcohol, no drugs.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Sharma et al.¹² (2019)</td>
<td>46</td>
<td>F</td>
<td>USA</td>
<td>Liver biopsy findings of steatohepatitis. Asymptomatic.</td>
<td>PEG precipitation</td>
</tr>
<tr>
<td>Lartigau-Roussin et al.¹³ (2017)</td>
<td>45</td>
<td>F</td>
<td>France</td>
<td>Appendectomy and depression; IgA myeloma; autoimmune thyroiditis; telangiectasias of the torso and back. Not taking therapy. Smoking and moderate alcohol.</td>
<td>GFC; immunofixation</td>
</tr>
<tr>
<td>Mbegaya et al.¹⁶ (2015)</td>
<td>44</td>
<td>F</td>
<td>UK</td>
<td>Epilepsy diagnosed at 12 years; constant widespread pain affecting limbs and trunk; poor sleep, irritable bowel and fatigue; not connective tissue disorder. Taking lamotrigine and sodium valproate.</td>
<td>PEG precipitation; GFC</td>
</tr>
</tbody>
</table>

No abnormality was reported in cardiac, abdominal, blood and serological tests (viral hepatitis B, C, A, Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus) as well as no fever, no myalgia, no jaundice, no night sweats, no weight loss, no pain or weakness in all patients. Therapy assumption has been reported.

macroAST, macro-aspartate aminotransferase; F, female; HBV, hepatitis B virus; Ig, immunoglobulins; PEG, polyethylene glycol; FANS, nonsteroidal anti-inflammatory drug; GFC, gel filtration chromatography; M, male; EBV, Epstein-Barr virus.
and there is heterogeneity of the macroAST molecule due to the different types of Ig or other plasma components. Finally, the PEG precipitation, described above, unlike the latter more complicated techniques, is a simple and low-cost method, which is commonly used for the detection of macroprolactin, possible in most routine laboratories and can be used for the screening of macroAST, giving a diagnosis in a short period of time. This method, however, has some limitations, like the possibility of interference with some assays, low specificity and the fact that an increase in serum globulins can lead to false positive results, as shown for macroprolactin.

Also, as shown in this case report, the fruitful cooperation and clear communication between clinicians and the clinical laboratory can lead to the early and correct diagnosis of macroAST, avoiding diagnostic confusion and unnecessary, time-consuming, expensive and even invasive investigations.  

Authors’ contribution
Maria Lorubbio wrote the manuscript. Agostino Ognibene, Benedetta Salvadori, Alessandra Fanelli and Giacomo Laffi critically re-viewed the manuscript. All authors participated in final approval of the manuscript.

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Conflicts of Interest
The authors have no conflicts to disclose.

REFERENCES
Instructions of Authors

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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.

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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 of 531 × 531 pixels (h × w) using a minimum resolution of 300 dpi. If you are submitting a larger image then please use the same ratio. Please note that your image will be scaled proportionally to fit in the available window, which is a 200 × 500 pixel rectangle.

Review articles

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

Editorials

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

Case reports

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

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

Letters to the editor

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

Special topics

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

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

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

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

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

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

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

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

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References should be numbered in the order they are cited, and the number of reference should be marked in the text by means of a superscript Arabic numerical. Only literature that is published or in press (with the name of the publication) may be numbered and listed; abstracts and letters to the editor may be cited. Cite the names of all authors when there are six or less; when seven or more list the first six followed by et al.
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Standard abbreviations not requiring definition are those listed in the Journal of Clinical Investigation. Otherwise, do not abbreviate unless a term is used more than five times in a paper. In this case, the abbreviation should be spelled out, in its first use in the text with the abbreviated form in parentheses, and it should also be listed on the footnote page (see above). Abbreviations used in figures or tables should be defined in the legend. Radiation measurements and laboratory values should be in accordance with the International System of Units (SI) (resources: "SI Units in Radiation Protection and Measurements, NCRP Report no. 82" [August 1985]; "Now Read This: The SI Units Are Here," JAMA 1986;255:2329-2339).

12. Drug names

Use generic names. The proprietary name may be mentioned in parenthesis. The names and locations (city and state or country) of manufacturers should be included in parentheses when mentioning proprietary drugs, tools, instruments, software, etc.
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3) Introduction, Methods, Results, Discussion, Acknowledgements, Conflict of Interest Statement, References

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