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Hepatitis C resistance to NS5A inhibitors: Is it going to be a problem?

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Abstract

Treatment of hepatitis C virus (HCV) infection has evolved greatly through the recent decade. The availability of direct-acting antiviral agents (DAAs) targeting the functional proteins of HCV has resulted in the introduction of DAA-based combination therapies, providing an optimal rate of treatment success. Among the DAAs, NS5A inhibitors are used in most of the introduced and approved HCV antiviral regimens. Resistance-associated substitutions (RASs) are amino acid substitutions in HCV protein sequences that result in decreased antiviral efficacy of the HCV DAAs. Among the HCV RASs, the NS5A RASs were found to effectively modify and decrease treatment response to NS5A inhibitor-containing regimens. As a baseline predictor of treatment response, NS5A RAS draws attention for pretreatment testing in targeted patient groups. Given NS5A RASs are either naturally-occurring or DAA-selected, the application of NS5A RAS testing can be considered in two settings of NS5A inhibitor-naïve patients and NS5A inhibitor-experienced patients. Less than 5% of NS5A inhibitor-naïve patients harbor naturally-occurring NS5A RAS with high resistance level (> 100X resistance fold-change). In NS5A inhibitor-naïve patients, NS5A RAS testing accompanied by treatment optimization cannot increase treatment response more than 2%-3%, while in NS5A inhibitor-experienced patients, > 75% are found to have NS5A RASs > 100X and NS5A RAS testing in this group of patients seems to be reasonable. This editorial will address the debate on the application of NS5A RAS testing and will discuss if the NS5A RAS testing has any role in clinical management of hepatitis C.

Key words: Direct-acting antiviral agent; Hepatitis C; NS5A; Resistance; Treatment

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Core tip: Hepatitis C virus resistance to NS5A inhibitors

is one of the main problems of treatment with NS5A inhibitors. While the treatment of NS5A inhibitor-naïve patients is feasible and efficient, retreatment of NS5A inhibitor-experienced patients is challenging. In the context of failure following treatment with NS5A inhibitor-containing regimens, NS5A resistance-associated substitution testing can help in clinical decision-making, while the usefulness of baseline NS5A resistance-associated substitution testing in NS5A inhibitor-naïve patients is in question.

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INTRODUCTION

Antiviral therapy of hepatitis C virus (HCV) infection has been the mainstay of hepatitis C management since application of conventional interferon (IFN) was introduced as the first HCV antiviral agent in 1990s^[1,2]. HCV antiviral therapy has transformed greatly over time, from use of immunomodulatory agents, such as IFN, to use of direct-acting antiviral agents (DAAs), such as NS3 protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors^[1]. While the IFN-based regimens result in suboptimal (30%-70%) virologic response, the DAA-based all-oral regimens have efficacy of > 95% in most patient groups^[3-5]. Moreover, the treatment response to IFN-based regimens was modified by a large number of different host and virus parameters, such as age, sex, host genetics, liver fibrosis, HCV ribonucleic acid (RNA) level, HCV genotype, variations in HCV genes, *etc*^[3,6,7].

On the other hand, with an increase of response rate to the DAA-based regimens, the modifiers of treatment response have been limited to few parameters, including cirrhosis, history of previous treatment (especially failure with DAA-based regimens), and finally naturally-occurring and DAA-selected resistance-associated substitutions (RASs)^[8,9]. Currently, in most of the approved regimens for treatment of HCV infection, NS5A inhibitors are one of the components of the combination therapy with DAAs. The currently available and approved NS5A inhibitors are ledipasvir (LDV), daclatasvir (DCV), ombitasvir, elbasvir, velpatasvir (VEL) and pibrentasvir (PIB). In the context of treatment with NS5A inhibitor-containing regimens, the NS5A RAS is one of the baseline predictors of treatment response and, also, the major finding after treatment failure with these regimens^[8,10].

NS5A RASs

Among the nonstructural proteins of HCV, the roles of NS5A protein are not fully known yet; however, it has

been found that NS5A binds the RNA-dependent RNA polymerase NS5B and modulates RNA-dependent RNA polymerase activity^[11]. The inhibitory activity of NS5A inhibitors on replication of HCV shows the pivotal role of NS5A protein in replication of HCV. The substitutions in the amino acid sequence of NS5A protein causing decreased binding of NS5A inhibitor molecules to NS5A protein can be selected under pressure of the treatment with NS5A inhibitors and subsequently decrease the inhibitory activity of NS5A inhibitors^[12].

Generally, the NS5A amino acid substitutions causing > 2.5 resistance fold-change are considered as NS5A RASs. A subset of these NS5A RASs cause > 100 resistance fold-change, and are considered NS5A RASs > 100X^[13]. It is worth noting that the resistance fold-change should be defined with consideration to the HCV genotype and subtype (*i.e.*, 1a, 1b, 3a, *etc.*), NS5A inhibitor type (*i.e.*, LDV, DCV, VEL, *etc.*) and also the specific amino acid substitution (*i.e.*, Y93H, Y93F, Y93L, *etc.*). The NS5A RASs are observed as naturally-occurring substitutions in a proportion (< 10% to > 50%) of NS5A inhibitor-naïve patients^[13-16]. The rate of detection of NS5A RASs is determined mainly by HCV genotypes and subtypes, and by the method for detection of NS5A RASs (*i.e.*, deep sequencing vs Sanger sequencing)^[17,18]. Among patients with NS5A RASs, only a small number (< 5% of NS5A inhibitor-naïve patients) of patients harbor isolates with naturally-occurring NS5A RASs > 100X.

The main finding regarding the prevalence of RASs in NS5A inhibitor-naïve patients with HCV genotype-1 (HCV-1) is the higher prevalence of NS5A RASs in patients with HCV-1b than in those with HCV-1a^[19,20]. In the study by Dietz *et al.*^[19], the prevalence of NS5A RASs was 7.1% in patients with HCV-1a infection and 17.6% in those with HCV-1b infection. These NS5A RASs could be found at different levels of a mixed population of the mutated and wild-type viruses^[21,22]. In NS5A inhibitor-experienced patients, especially those who have undergone a complete course of 12-wk or > 12-wk treatment, DAA-selected NS5A RASs are observed in > 75% of patients following treatment failure^[10,23-25]. Most of these patients with failure to NS5A inhibitor-containing regimens harbor isolates with NS5A RASs > 100X^[24,26]. Unfortunately, these DAA-selected NS5A RASs are persistent and they are observed even in the follow-up, 96 wk after treatment failure^[27].

The impact of naturally-occurring baseline NS5A RASs has been assessed in many studies. These studies showed the impact of NS5A RASs on treatment response to NS5A inhibitor-containing regimens such as LDV/sofosbuvir^[23,28], grazoprevir/elbasvir^[29-31], ombitasvir/paritaprevir/ritonavir^[21] and DCV/asunaprevir^[22]; however, treatment with regimens containing the second-generation NS5A inhibitors with medium resistance barrier, including sofosbuvir/VEL^[26,32] and glecaprevir/PIB^[33], was either not impacted or only minimally modified by the NS5A RASs. In terms of HCV genotypes, it seems that the treatment of patients with HCV-3 infection

using first-generation NS5A inhibitors is more influenced by NS5A RASs than that of patients with HCV-1 infection using same NS5A inhibitor^[13,26]. This finding can be a result of different resistance fold-change of NS5A RASs by HCV genotypes with higher resistance fold-change for NS5A RASs in HCV-3 isolates than that for NS5A RASs in HCV-1 isolates^[34]. Finally, it can be concluded that the higher response rate observed with the optimized treatment using a second-generation NS5A inhibitor with higher resistance barrier (*i.e.*, VEL, PIB, *etc.*) effectively inhibiting NS5A protein of all HCV genotypes (pan-genotypic regimen) results in a lower chance for observation of treatment response modification by NS5A RASs.

With the knowledge that most of the patients with failure following treatment with NS5A inhibitor-containing regimens harbor NS5A RASs, especially those which confer a high level of resistance, the impact of these DAA-selected NS5A RASs on the efficacy of retreatment were evaluated in a small number of studies; the results showed that NS5A RASs are prominent treatment response predictors, especially when the regimen of the retreatment was not intensified or optimized for eradication of the virus^[35,36].

TESTING RESISTANCE TO NS5A INHIBITORS: WHEN AND HOW?

There is a great debate on the application and usefulness of NS5A RAS testing prior to treatment. Generally, NS5A RAS testing can be considered under two different conditions: (1) prior to treatment with NS5A inhibitor-containing regimens in NS5A inhibitor-naïve patients; and (2) prior to retreatment with NS5A inhibitor-containing regimens in patients with a history of treatment failure using NS5A inhibitor-containing regimens. In Figure 1, the strategies with and without pretreatment NS5A RAS testing for treatment of HCV infection using NS5A inhibitor-containing regimens in NS5A inhibitor-naïve patients are presented. Since the overall response rate to the currently available and standard NS5A inhibitor-containing regimens is high (> 95%) and also the prevalence of NS5A RASs conferring high resistance level is low (< 5%), the strategy of pretreatment NS5A RAS testing and optimization of treatment in terms of treatment prolongation, adding ribavirin (RBV) and targeting all three HCV protease, NS5A and polymerase proteins can result in 2%-3% increase of the overall treatment response rate. Considering the costly (\$100-\$500) and time-consuming (7-30 d) process of NS5A RASs testing, we do not recommend NS5A RAS testing in NS5A inhibitor-naïve patients prior to treatment with NS5A inhibitor-containing regimens.

In patients with previous history of treatment with NS5A inhibitor-containing regimens, the scenario is much different with the fact that > 75% of them

harbor NS5A RASs. As shown in Figure 2, the NS5A RAS testing can be implemented in the management of patients with previous history of treatment with NS5A inhibitor-containing regimens. If the patient harbors the NS5A RAS, they should be treated with the most intensified and optimized available treatment regimen (adding RBV, prolongation of treatment to 24 wk, and targeting all three protease, NS5A and polymerase proteins). However, the small portion of patients with previous history of treatment with NS5A inhibitor-containing regimens and without detectable NS5A RAS can be treated by treatment prolongation and/or adding RBV without targeting additional HCV protein.

Currently, there are services available for NS5A RAS testing in diagnostic laboratory centers. These services are based on Sanger direct population sequencing or deep sequencing using next-generation sequencing platforms. The main difference between these two available technologies is the limit of detection of the RAS (mutated nucleotide) among the population of the virus without the RAS (wild-type nucleotide). Sanger sequencing can detect the NS5A RASs comprising 20%-30% of the whole population of the virus, while deep sequencing can even detect NS5A RASs comprising < 1% of the whole HCV population. It figures that deep sequencing is much more advanced for detection of NS5A RASs than Sanger sequencing; however, it was found that a small (< 10%-20% of the whole genetic pool of virus) population of HCV with RAS in a patient has no or minimal impact on treatment success^[21,22,37]. With this finding, it seems that in the clinical management of hepatitis C, the NS5A RAS testing using Sanger sequencing is reasonable, and the reports of NS5A RAS testing using deep sequencing methods should be accompanied by the percentage of the NS5A RAS in the whole virus population.

CONCLUSION

DAA-based treatment of HCV is one of the greatest recent achievements in the field of hepatology and liver diseases, making hepatitis C a curable disease; however, the main problem with most of the targeted therapies is resistance. With a combination of NS5A inhibitor with an NS5B polymerase inhibitor and/or an NS3 protease inhibitor, the problem of HCV resistance has been mostly resolved in DAA-naïve patients and only a small (< 5%) population of treated patients relapsing after treatment termination. While pretreatment NS5A RAS testing cannot help in management of DAA-naïve patients, it can help in treatment decision-making for patients with failure to NS5A inhibitor-containing regimens. Currently, with the introduction of second-generation NS5A inhibitors, VEL and PIB, and the upcoming NS5A inhibitors in the pipeline with a high barrier to resistance, the problem of resistance to NS5A inhibitors is going to fade. However, unsolved concerns remain, such as transmission of NS5A RASs in populations with high-

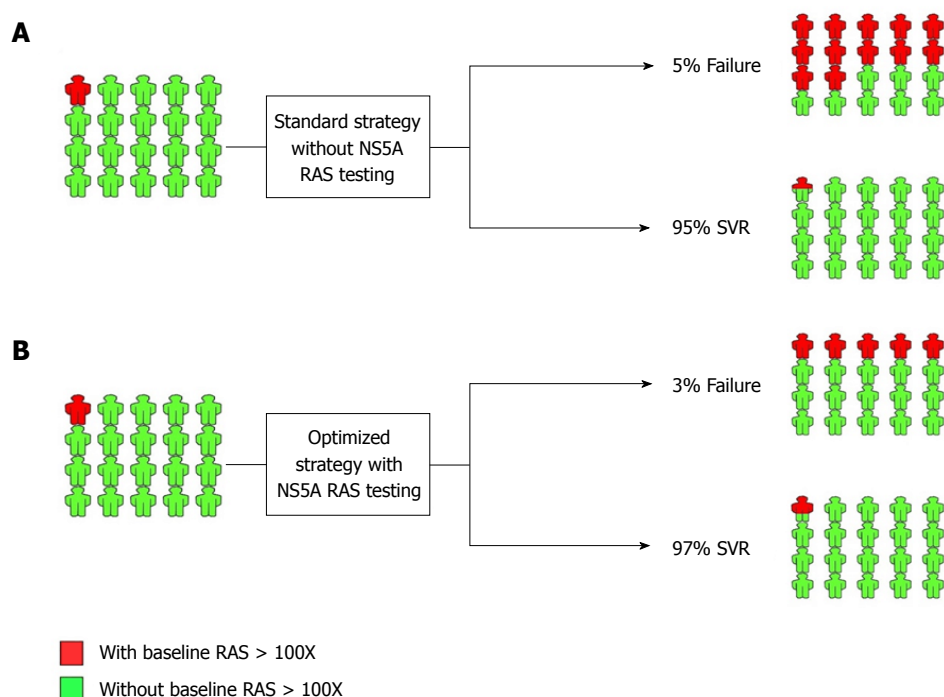


Figure 1 NS5A resistance-associated substitution testing in NS5A inhibitor-naïve patients prior to treatment with NS5A inhibitor-containing regimen. A: Standard treatment strategy and outcome without NS5A resistance-associated substitution (RAS) testing; B: Optimized treatment strategy and outcome with NS5A RAS testing. The implementation of NS5A RAS testing is developed by decision-making based on the presence of RAS > 100X. SVR: Sustained virologic response.

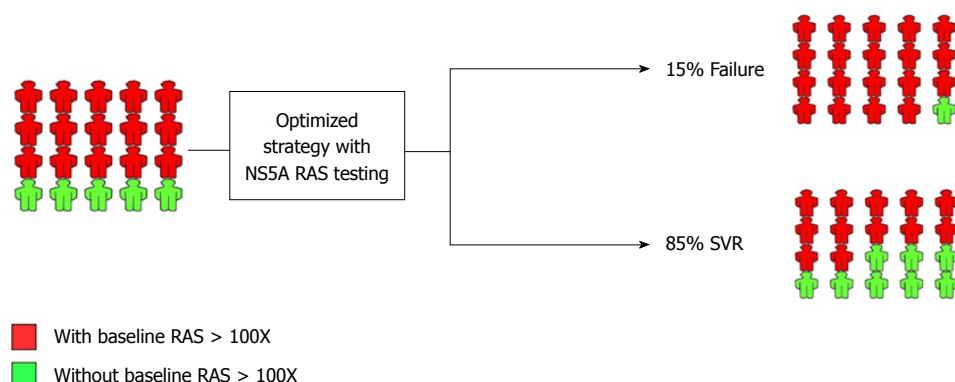


Figure 2 NS5A resistance-associated substitution testing in NS5A inhibitor-experienced patients prior to retreatment with NS5A inhibitor-containing regimen. Optimized treatment strategy with NS5A resistance-associated substitutions (RAS) testing and the outcome is shown. The implementation of NS5A RAS testing is developed by decision-making based on the presence of RAS > 100X. SVR: Sustained virologic response.

risk behaviors, including people who inject drugs, which should be considered by clinicians and researchers carefully.

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Challenge of hepatitis C in Egypt and hepatitis B in Mauritania

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Abstract

Egypt has one of the highest prevalence rates of hepatitis C virus (HCV) in the world, mostly with genotype 4 that is highly associated with severe fibrosis. As a consequence, hepatocellular carcinoma has become the leading cause of cancer in this country. Mauritania is a highly endemic area for hepatitis B virus (HBV). HBV and HCV could both be iatrogenically transmitted through infected blood products, infected needles, and medical equipment improperly sterilized. Adequate and efficient healthcare and public health measures with good surveillance programs, access for screening, prevention strategies, and successful treatment are needed to halt the spread of these diseases. Herein, we have reviewed the epidemiology, modes of transmission, predisposing factors, and novel treatment modalities of these viruses. We have proposed practices and interventions to decrease the risk of transmission of HCV and HBV in the affected countries, including strict adherence to standard precautions in the healthcare setting, rigorous education and training of patients and healthcare providers, universal screening of blood donors, use of safety-engineered devices, proper sterilization of medical equipment, hepatitis B vaccination, as well as effective direct-acting antiviral agents for the treatment of HCV.

Key words: Hepatitis B virus; Hepatocellular carcinoma; Hospital acquired infection; World Health Organization; Hepatitis delta virus; Hepatitis C virus

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Core tip: Hepatitis C virus (HCV) and hepatitis B virus (HBV) are major public health concerns in Egypt and Mauritania. HCV and HBV can both be transmitted through medical and surgical procedures (healthcare-associated transmission) among others. Screening, prevention, and treatment strategies should be emphasized in Egypt and Mauritania to prevent the spread of these diseases. Direct-acting antivirals for the treatment of HCV are highly effective and well tolerated and should be made accessible and affordable to patients.

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HEPATITIS C VIRUS IN EGYPT

Over the last several years, Egypt continues to have one of the largest epidemics of hepatitis C virus (HCV), with an estimated prevalence of 10%. A prevalence above 4% is considered high by World Health Organization (WHO) standards^[1]. A study published in the Proceeding of the National Academy of Science (PNAS) reports more than 500000 new HCV cases yearly^[2]. However, others recently estimated a lower number of new cases every year, but still it remains high compared to other areas in the world^[3]. Hence, viral hepatitis caused by HCV genotype 4 continues to represent the most serious public health threat currently facing Egypt^[1,2].

To estimate the prevalence of HCV in Egypt, a national survey was conducted in 2015 known as the Egyptian Health Issues Survey (EHIS). This survey was a cross-sectional household survey of 16003 who were identified between the ages of 15 and 59 and had blood testing for HCV^[1]. The results of this survey were compared to another cross-sectional national survey conducted in 2008 known as the Egyptian Demographic Health Survey (EDHS) in which 12008 Egyptian were interviewed^[4]. The 2015 survey (EHIS) reported a prevalence of 10% for HCV antibody and 7% for HCV RNA. This reflected an estimated 29% reduction in HCV RNA prevalence compared to the 2008 national survey (EDHS). This reduction could be related to the disappearance of the highly infected group that was treated with reused syringes during the schistosomiasis treatment campaign in the 1960s and 1970s^[1]. Hence, by 2015, this highly infected older age group

disappeared due to differential age related migration and mortality (particularly since the survey excluded individuals that are 60 years or older). Furthermore, when a shift adjustment was made (by 7 years), the age specific prevalence of HCV RNA positivity was matched and was comparable in the 2008 surveillance (EDHS) and the 2015 surveillance (EHIS). Hence, these surveys represent an underestimate of the true prevalence of the HCV infections in Egypt, particularly since the rate of infection is among elderly patients above 60 years who acquired the infections in the 1960s and 1970s whereas these surveys were limited only to individuals with an age range of 15-59 years^[1].

Based on the above and given the population of Egypt, which exceeds 95 million, we estimate that more than 6.5 million Egyptians are infected with the HCV virus, and most of those are caused by a genotype 4. Several genotypic studies have shown that genotype 4 of HCV accounts for 93.1% of HCV infections, and most (80.6%) of the Egyptians infected with HCV4 belong to subtype 4A^[5-7]. It is likely that the subtype 4A was the main strain associated with antischistosomal therapy epidemic that occurred in the 1960s and 1970s and which was associated with the reuse of needles^[8,9]. In fact HCV4 is the predominant HCV genotype in the Middle East and North Africa, which accounts for 59% of HCV infections in Syria, 53% in Iraq, 54% in Kuwait, and 64% in Palestine. It is also reported to be a dominant genotype in Qatar, Saudi Arabia, and Libya^[5,6,10,11] and is common in other parts of the Middle East such as Lebanon, Oman, and UAE. This may possibly be related to the migration of Egyptians, who represent a major workforce in some of these Middle Eastern countries, to these areas^[5,11].

Furthermore, there is evidence that HCV4 is a highly pathogenic virus. Wali *et al*^[12] demonstrated that HCV4 was significantly more associated with fibrosis progression, severe fibrosis development, and confluent necrosis than other non-genotype 4 HCV infected patients. In addition, hepatocellular carcinoma (HCC) in Egypt is a leading cause of cancer and cancer mortality among men, whereby more than 84% of Egyptian patients with HCC are positive with HCV4^[13,14]. Chronic infection that could lead to severe fibrosis and cirrhosis can occur in 50%-85% of HCV patients^[15,16]. Given the decline in the economic and healthcare conditions, nationwide efforts to control the spread of HCV infection have been disrupted for several years.

The iatrogenic spread of HCV genotype 4 in Egypt due to the improper dental instruments sterilization has been a major concern^[17]. Furthermore, between 46%-100% of hemodialysis patients and 11%-82% of patients who received multi-transfusions were found to be seropositive for HCV^[18].

Despite all the effort done by the Ministry of Health and Population (MOHP) in Egypt to control infection, healthcare-associated infection (HAI) remains one of the most common cause of HCV infection in Egypt,

Table 1 Risk factors of the transmission of hepatitis C in Egypt through the healthcare system and proposed interventions

Risk factors	Proposed interventions
Needle stick injuries or other injuries	Institute infection control and occupational health programs in all healthcare facilities to reduce occupational exposure, protect against needle stick, and other healthcare related injuries Adequate education and training of healthcare providers Use of safety-engineered devices, such as needleless intravenous medication systems, blunted suture needles Use of needle disposal containers
Surgical or invasive interventions, dental procedures	Appropriate sterilization of surgical and dental instruments Good aseptic techniques practiced during invasive procedures Provide personal protective equipment, such as gloves, gowns, face/eye shields, to be used during procedures with anticipated blood exposure
Exposure to medical equipment, hemodialysis machines and procedures	Strict infection control and prevention policies Universal precautions should be used when caring for all patients
Injection and IV insertion	Use of self-sheathing needles, needleless connectors, needleless intravenous medication system, and needle disposal containers
Blood transfusion from poorly screened individuals (false negative anti-HCV)	Universal screening of all donors
Organ donation	Universal screening of all donors

HCV: Hepatitis C virus.

related to the overuse of contaminated needles and syringes^[2]. In addition, healthcare workers in Egypt and many Middle Eastern countries have the highest rates of needle stick injuries worldwide^[19,20]. Hence, unsafe medical and dental practices, including reuse of medical devices, inadequate sterilization of surgical and interventional equipment, poor aseptic techniques practiced during invasive procedures, circumcisions or deliveries of neonates by providers, unsafe injections, and limited testing of blood product transfusions for HCV have all contributed to high iatrogenic transmission of HCV in Egypt^[21-23]. These factors play an important role in the transmission of HCV in Egypt. Table 1 outlines the risk factors for the transmission of HCV in Egypt through the healthcare system, and the proposed infection prevention interventions that could control this transmission.

To control this self-perpetuating HCV epidemic in Egypt, a health initiative was started in 2017 to screen all governorates of Egypt called "Egypt without virus C, 2020" in cooperation with Tahya Misr Fund^[24]. A concerted effort should be made towards universal screening of all patients going through the healthcare system, early detection of HCV, early treatment using direct acting antiviral (DAA) regimen with simultaneous implementation of strict infection control and prevention policies within the healthcare system.

Egypt also developed a strategy for prevention and control of viral hepatitis (2008-2014) in collaboration with WHO, Centers for Disease Control (CDC), and Pasteur^[25]. The National Committee for Control of Viral Hepatitis (NCCVH) in affiliation to the MOHP established a large model of care in Egypt since 2006 that aimed at elimination of HCV in Egypt and delivering DAAs for all patients at the expense of the government^[25,26]. However, the limited resources, the unmet needs and suboptimal access to care, and the low rates of patients'

follow-up hindered the success of the program^[27,28].

Most of the individuals living with viral hepatitis caused by HCV are asymptomatic and, hence, remain unaware of their illness for decades, even though liver damage is occurring. Given the high prevalence of HCV in Egypt, universal surveillance through rapid enzyme immunoassay testing of all patients going through the healthcare system would detect a large number of asymptomatic patients. With the advent of DAA and the demonstration of high cure rates (sustained virologic response or SVR) of over 90%, including genotype 4 infected patients. The DAAs are also associated with minimal side effects and relatively short duration of treatment, when compared to interferon based treatments.

Hence, it is possible to virologically cure the majority of these patients, particularly if the patient is treated in the early phase when they are not suffering from decompensated liver disease or cirrhosis^[29-32]. Curing these patients would preempt the morbidity and mortality associated with subsequent liver cirrhosis and HCC. SVR after non-interferon DAA regimens is associated with improvement in liver fibrosis and necrosis in up to 73% of patients, with reversal of cirrhosis in 49% of the cases^[33]. Emerging data with DAAs show significant improvement of liver stiffness that has been reported in patients with HCV-associated advanced liver disease^[34]. A recent study showed that DAA-induced SVR was associated with 71% reduction in HCC^[35].

Early treatment of HCV infection, regardless of the infecting genotype, may also reduce the risk of extrahepatic complications, including mixed cryoglobulinemia, porphyria cutanea tarda, diabetes mellitus, cardiovascular disease, renal diseases, and B-cell non-Hodgkin lymphoma^[36-38].

Given the availability of generic versions of DAAs

(particularly sofosbuvir at an affordable low cost), early treatment can be widely used and, hence, could effectively contribute to the elimination of HCV viral hepatitis in Egypt^[3]. The WHO reported that after generic sofosbuvir became available in Egypt at a cost of \$153 for a 12-wk course, approximately half a million patients with chronic HCV were treated with this drug since January 2016^[3,39]. Our team conducted a large surveillance study on patients evaluated at Harpur Memorial Hospital in the Delta area of Menouf, Egypt, involving 729 adult patients (18-65 years) who were evaluated for health-related illnesses not associated with hepatitis or liver diseases between January 2012-January 2013. All patients who consented were screened by a rapid enzyme immunoassay (ELISA) to detect the presence of HCV antibodies and determine the prevalence of HCV (HCV antibodies). The reactive ELISA rapid test samples were further confirmed for HCV antibody positivity by the chemiluminescent microparticle immunoassay (CIA). Subsequently, CIA HCV antibody positive samples were tested further for HCV RNA by quantitative real time polymerase chain reaction (PCR). We identified 146 patients (20%) who were positive for HCV, which was later confirmed by the ELISA antibody test and HCV-RNA levels (viral load). Interestingly, 119 (82% of the 146 patients) with a positive test had no risk factors for developing HCV, such as a surgical procedure, dental extraction, needle stick injuries, blood transfusions, and other risk factors listed in Table 1 in the prior 5 years. All of the 146 patients with positive HCV in the study were asymptomatic with no symptoms of liver disease. Therefore, this study highlights the importance of universal surveillance in the general population, as suggested by WHO, in countries with high HCV antibody seroprevalence ($\geq 2\%$), which will lead to the early detection of HCV in asymptomatic patients and ultimately lead to high cure rates after using DAA^[29-32,40].

In addition to universal surveillance, early diagnosis, and early treatment of HCV in Egypt, promotion of infection prevention and control procedures in a healthcare setting is of paramount importance in controlling HCV transmission^[40]. These infection control policies and practices should prohibit reuse of medical devices or needles, emphasize appropriate sterilization of surgical and dental instruments, promote the use of safe injectables, protect against needle stick and other healthcare related injuries, test all blood donors, and adhere to appropriate healthcare waste management. This involves a large scale training of healthcare professionals in Egypt on infection control practices and includes new safeguard technologies, such as the auto-disable syringes (Table 1).

The conventional combination regimen of pegylated-interferon and ribavirin alone has demonstrated low SVR rates against genotype 4 of approximately 30% after 48 wk of treatment with poor tolerance of the regimen^[41,42]. However, several DAAs have been approved in the United States and Europe that showed

highly promising results; and, hence, they have changed the treatment paradigm for chronic HCV infections in general, including genotype 4 (Table 2)^[43,44]. The DAA regimen should be individualized, mostly on the basis of the patient's prior antiviral therapy, presence of cirrhosis, availability, cost, and drug-drug interactions with concomitant medications^[43]. In most of these regimens, the treatment duration extended between 12-24 wk^[29-32,43-45].

One of the first interferon-free regimens used in treatment of genotype 4 HCV, which consisted of sofosbuvir plus ribavirin, showed that 12 wk of treatment was associated with overall lower SVR rates compared to 24 wk, and, hence, this regimen was approved for 24 wk^[29].

In particular, several fixed dose combination regimens, such as ledipasvir-sofosbuvir^[30,46,47], sofosbuvir-velpatasvir^[31,48], and glecaprevir/pibrentasvir^[49,50], have demonstrated efficacy with SVR that exceeds 90% after 8-12 wk of treatment of HCV 4. They are unique in that they achieve successful outcome with once daily dosing and without the need to administer ribavirin in the treatment of genotype 4 HCV. Only glecaprevir/pibrentasvir can be used for 8 wk in the subset of treatment-naïve or peginterferon/ribavirin-experienced genotype 4 patients without cirrhosis^[43,49,50].

Ledipasvir-sofosbuvir is available and widely used in the treatment of genotype 4 HCV patients in Egypt. In an open label phase 2 trial, Kohli *et al.*^[30] showed that ledipasvir-sofosbuvir treatment of patients with HCV genotype 4 for 12 wk resulted in 100% SVR 12 and was well tolerated with minimal mild adverse events. Crespo *et al.*^[47] reported on the effectiveness and safety of this once daily oral combination in the treatment of hepatitis C genotype 4 infections, showing an overall 95.4% SVR 12 response with a respective 100% SVR 12 response in patients without cirrhosis. In patients with cirrhosis, 12-wk treatment with ledipasvir-sofosbuvir without ribavirin had an equivalent successful outcome to this once daily combination with ribavirin for 12 wk or 24 wk^[47]. However, many of the genotype 4 HCV patients in Egypt are elderly patients with some renal insufficiency or reflux disorder requiring proton pump inhibitors (PPIs), which might limit the activity and the use of the drug in this population^[51,52].

Sofosbuvir-velpatasvir has also demonstrated high efficacy in the treatment of patients with genotype 4 HCV infections irrespective of treatment history (treatment naïve or experienced) or the presence of cirrhosis^[31,48]. In a study by Feld *et al.*^[31], 100% of the 116 patients with genotype 4 infection achieved SVR 12 following a 12-wk treatment of sofosbuvir-velpatasvir. Similarly, this regimen was highly effective in genotype 4 HCV infections and was well tolerated, with serious adverse events occurring in only 2% of those who received this regimen^[48].

The fixed dose combination of glecaprevir-pibrentasvir given once daily in three pills was uniquely effective in the treatment of HCV genotype 4 infected

Table 2 Direct-acting antiviral regimens available to treat hepatitis C virus genotype 4

¹ Combination regimen ^[43,44]	Duration (wk)
Sofosbuvir-ledipasvir	12
Sofosbuvir-velpatasvir	12
Glecaprevir-pibrentasvir	8 (without cirrhosis) 12 (with cirrhosis)
Sofosbuvir-velpatasvir-voxilaprevir	12
Ombitasvir-paritaprevir-ritonavir ± ribavirin	12
Elbasvir-grazoprevir ± ribavirin	12-16
Elbasvir-grazoprevir	12 (treatment naïve)
Elbasvir-grazoprevir + ribavirin	16 (treatment experienced)
Sofosbuvir + ribavirin	24
Sofosbuvir + daclatasvir ± ribavirin	12
Sofosbuvir + simeprevir ± ribavirin	12-24

¹The information from the table is from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) hepatitis C virus guidelines.

patients when given for only 8 wk in patients without cirrhosis, resulting in an SVR 12 response rate of 93% in this patient population^[49,50]. Furthermore, in 16 patients with cirrhosis who received 12 wk of this fixed combination, 100% SVR 12 was achieved. It is important to note that the HCV genotype 4 patients without cirrhosis who did not achieve SVR 12 with the 8 wk regimen were either patients who had incomplete data or discontinued therapy prematurely^[50]. The advantage of this fixed dose combination regimen of glecaprevir-pibrentasvir is that it can be given in patients in any degree of renal impairment, unlike ledipasvir/sofosbuvir, sofosbuvir/velpatasvir, and sofosbuvir/velpatasvir/voxilaprevir^[49,50].

HEPATITIS B IN MAURITANIA

Hepatitis B virus (HBV) is a major cause for liver disease worldwide, particularly in Mauritania^[53]. There are approximately two billion people who have been infected with HBV, of which 400 million people are infected chronically and of whom 65 million reside in Africa. HBV infection leads to 0.5-1.2 million deaths annually due to liver cirrhosis and HCC. Sub-Saharan Africa has a high HBV prevalence rate of 16.16%^[54,55]. Mauritania remains a hyper-endemic area for HBV. Studies in Mauritania on the epidemiology of HBV^[56] have shown a high prevalence of HBsAg positivity in blood donors, mostly between the age of 21-30, despite HBV vaccination of children and newborns since 2000^[57]. Moreover, high HBV DNA levels were shown to be associated significantly and independently with the incidence of cirrhosis and HCC.

Co-infection with hepatitis delta virus (HDV)^[58] is also endemic in Mauritania^[59]. There is a high prevalence of HBV (20%) and HDV (30%). HDV infection tends to occur early, affecting mainly children and young adults, leading to chronic hepatitis. The natural course of chronic HDV is rapid progression to cirrhosis and liver related complications, including HCC^[60,61].

The modes of transmission in Mauritania for HBV

and HDV are social close contact, sexual contact, sharing needles, and other forms of blood exchange as well as maternal-fetal transmission during delivery (Table 3). Interfamilial transmission is common and may be facilitated by poor hygiene. Thus, socially and economically disadvantaged populations, as found in Mauritania, are more affected. Children infected perinatally with HBV are asymptomatic, and 25% die in adulthood from cirrhosis complications or HCC^[62].

HCC remains a major cause of mortality due to limited therapeutic options. The aim should focus on prevention. Effective vaccine for HBV is available and was recommended by WHO to be included in the national immunization program. However, not all countries have adopted and implemented this recommendation effectively, including Mauritania, which ranks among the countries with the highest mortality of HBV associated HCC^[62].

Hepatitis B vaccination is the best strategy to prevent this infection and decrease its incidence in the young population^[63]. Vaccination is highly recommended in high prevalence areas like Mauritania, and the vaccines should be given to all infants at the time of birth, children, and adolescents as well as adults with risk factors that are included in Table 3. In addition, booster doses of hepatitis B vaccine are recommended in hemodialysis and in the immunocompromised person. The high cost of the vaccine and the lack of the infrastructure to deliver the vaccines do impact the implementation of a universal vaccination program in Mauritania. Prevalence rates of hepatitis B and children vaccination rates in various African region have been published^[64]. However, given the limited data available from the unaccounted home births, the reported vaccination rates could be inflated and the prevalence of the disease understated. Therefore, public health action is urgently needed. A multifaceted approach to improve the socioeconomic conditions, increase the awareness of the risk of transmission, aggressive vaccination campaigns, and public health intervention are strongly needed to prevent viral transmission. Also, a regional

Table 3 Risk factors of transmission of hepatitis B in Mauritania and proposed interventions

Risk factors	Proposed interventions
Direct contact with infected blood and or handling blood or body fluids (job exposure)	Rigorous adherence to standard precautions in healthcare settings Completely avoid sharing needles or re-using disposable devices
Sharing needles or other equipment (such as cotton, spoons, and water) to inject drugs	Education of healthcare providers and patients Hepatitis B vaccinations and assessment of response to vaccine (hepatitis B surface antibody)
Hemodialysis	Use of safety-engineered devices and needless infusion systems Use of sharp object disposal containers Strict infection control measures upon cleaning and reusing medical equipment Appropriate screening of blood donors Post-exposure prophylaxis Antiviral therapy
Intimate contact with a person with HBV	Hepatitis B vaccination Avoid sharing toothbrushes, razors, <i>etc.</i>
Multiple sex partners or having unprotected sex with someone who is infected with the virus	Hepatitis B vaccination Protected sexual intercourse
Mother-to-Child transmission	Screening pregnant women Antiviral therapy to pregnant women with high DNA levels Passive-active immunization of newborns of mothers with HBV Universal vaccination of newborns
Body piercings, tattoos or acupuncture	Avoid body piercing and tattoos Strict infection control and prevention policies
IV drug users	Avoid sharing syringes and needles

HBV: Hepatitis B virus.

specific clinical guideline for screening, targeting infection control measures, and using ultra-sonographic tools in the highest risk setting, such as healthcare, dentistry, and personal grooming service centers, is needed. In addition, enhanced access to health care services and providing public funds for treating HBV and HDV may help to optimize management of infected patients.

Patients with acute hepatitis B do not require antiviral medication, as most patients will recover spontaneously. Supportive care with hydration and regular follow up with their physician is recommended. However, patients with chronic hepatitis B are more likely to require antiviral medications. Its purpose is to stop any further damage to the liver by slowing the multiplication of the virus. Therapy should be given to the following patients: patients with chronic hepatitis B with HBV DNA > 20000 IU/mL and with liver alanine aminotransferase > 2 × upper limit of normal, patients with evidence of fibrosis or moderate to severe hepatitis on liver biopsy irrespective of hepatitis B DNA level, and patients with cirrhosis associated with chronic HBV irrespective of hepatitis B DNA level.

Nucleoside analogues are the preferred agents. They are very effective at viral suppression and have a high barrier to drug resistance. The current first line therapy are entecavir 0.5 mg/d × 48 wk orally, tenofovir 300 mg/d × 48 wk orally^[65], and pegylated interferon in noncirrhotic HbeAg-positive patients is also an option^[66]. However, most patients require lifelong therapy because relapse is common after discontinuation of therapy^[67,68]. Unfortunately, most of Mauritians with chronic HBV infection who are eligible for this therapy cannot afford

it. Less than 1% of individuals eligible for antiviral therapy receive HBV treatment. In mothers with a high viral load, the antiviral treatment rate to reduce mother-to-child transmission is even lower^[69]. In summary, HCV and HBV are major causes of morbidity and mortality worldwide. Both could be iatrogenically transmitted through infected blood products, infected needles and medical equipment. Egypt has one of the highest prevalence rates of hepatitis C in the world and North Africa which could be controlled by rigorous infection control measures with education of healthcare providers and patients as well as universal screening of blood donors. In addition providing the current efficacious antiviral therapy (DAA) for the Egyptians infected with HCV could also help eliminate this infection. On the other hand, Mauritania is a highly endemic area for hepatitis B and ranks among the North African countries with the highest mortality of HBV and associated HCC. Despite this impressive burden, hepatitis B in Mauritania is now considered a preventable disease with the available vaccine and the proposed implementation of several infection prevention measures. Universal surveillance programs, active infection prevention strategies particularly within the healthcare system and early detection with early treatment and precaution could be the common strategy that will halt the spread of both HCV and HBV diseases in these two countries.

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Micro-RNAs in hepatitis B virus-related chronic liver diseases and hepatocellular carcinoma

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Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that modulate gene expression at the post-transcriptional level by affecting both the stability and translation of complementary mRNAs. Several studies have shown that miRNAs are important regulators in the conflicting efforts between the virus (to manipulate the host for its successful propagation) and the host (to inhibit the virus), culminating in either the elimination of the virus or its persistence. An increasing number of studies report a role of miRNAs in hepatitis B virus (HBV) replication and pathogenesis. In fact, HBV is able to modulate different host miRNAs, particularly through the transcriptional transactivator HBx protein and, conversely, different cellular miRNAs can regulate HBV gene expression and replication by a direct binding to HBV transcripts or indirectly targeting host factors. The present review will discuss the role of miRNAs in the pathogenesis of HBV-related diseases and their role as a biomarker in the management of patients with HBV-related disease and as therapeutic targets.

Key words: Hepatitis B virus infection; MicroRNAs; Hepatitis B virus pathogenesis; Molecular mechanisms

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Core tip: This review article will focus on the emerging puzzle of hepatitis B virus (HBV)-hepatocyte interaction *via* miRNAs, indirectly or directly modulating HBV

replication and pathogenesis, and thus on the role of microRNAs in the natural history of HBV infection. We evaluated the literature on their possible future role as a biomarker in the management of patients with HBV-related disease and as therapeutic targets.

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INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNAs that modulate gene expression at the post-transcriptional level by affecting both the stability and translation of complementary mRNAs^[1]. MiRNAs play crucial roles in a variety of physiological processes, such as cell development and differentiation^[2,3]. miRNA mutations, dysregulation of their expression or dysfunction of miRNA biogenesis lead to an interference with biological pathways involved in the development and evolution of human diseases, including cancer, cardiovascular diseases and infectious diseases^[4-8].

About the host-virus interplay, various studies have shown that miRNAs are important regulators in the conflicting efforts between the virus (to manipulate the host for its successful propagation) and the host (to inhibit the virus), culminating in either the elimination of the virus or its persistence. In fact, different viruses encode miRNAs that modulate not only the viral mRNA expression to regulate its own lifecycle, but also the host mRNA expression to establish a cellular environment resulting favorable to their replication; on the other hand, host cells encode miRNAs counteracting viral replication^[8-10]. An increasing number of studies report a role of miRNAs in hepatitis B virus (HBV) replication and pathogenesis. HBV is a non-cytopathic virus belonging to the Hepadnaviridae family. It has a 3.2 kb partially double-stranded DNA, showing 4 known open reading frames (ORFs): The S region, which contains three in-frame initiator codons, codes for the small, medium and large surface antigen (HBsAg) proteins; the C region, with two initiator codons for the core and "e" antigen (HBcAg, HBeAg); the P frame, coding for an RNA-dependent DNA polymerase; and the X region, coding for a protein regulating the transcription of both viral and cellular genes^[11].

The present review will discuss the emerging puzzle of HBV-hepatocyte interaction *via* miRNAs, indirectly or directly modulating HBV replication and pathogenesis, and thus will focus on the role of microRNAs in the natural history of HBV infection and on their possible

future role as biomarkers in the management of patients with an HBV-related disease and as therapeutic targets.

HBV INFECTION

Despite the universal vaccination campaigns against HBV infection undertaken in many countries over the last two decades, this infection remains a global health problem. In 2015, the WHO estimated that nearly 3.5% of the world population live with chronic HBV infection^[12], with about 800000 deaths per year (460000 for complications of liver cirrhosis and 340000 for hepatocellular carcinoma-HCC)^[13]. For non-immune/non-infected subjects any parenteral or mucosal exposure to blood, blood products or blood-contaminated material should be considered a risk for acquiring HBV infection^[14]. In addition, being present in semen and cervical secretions at infectious concentrations, HBV is also transmitted by sexual and vertical routes^[15]. The age at the time of infection strongly modulates the progression to chronicity, which occurs in around 90% of subjects infected at birth, a rate progressively decreasing with the increase in age at infection, up to 2%-5% in the adult population^[16].

The geographical distribution of HBV chronic carriers is highly variable, ranging from 0.7%-1% in developed western countries to 8% or more in some countries in sub Saharan Africa and South-East Asia, depending on environmental factors, earning potential, educational levels and lifestyles. In countries with an intermediate-high level of endemicity, HBV infection is most frequently acquired at birth from an HBeAg-positive mother or through horizontal transmission in early childhood by household contacts (most frequently between siblings); in these cases the rate of progression to chronicity is high, which keeps the prevalence of infection in these geographical areas intermediate or high. Intravenous drug addiction is the major risk factors for acquiring HBV infection in countries with a low HBV endemicity like Western Europe^[15] and North America^[17], whereas promiscuous unprotected sexual activity is a main risk factor worldwide.

The clinical presentation of chronic HBV infection is variable, ranging from asymptomatic carriage of the virus to liver cirrhosis with or without HCC^[18]. Patients with chronic hepatitis develop liver cirrhosis with an incidence of 1-5 per 100 persons/year, with a 5-year cumulative probability of progression ranging from 8% to 20%, depending on the degree of disease activity, HBeAg/anti-HBe status, the HBV load and co-morbidities^[19]. The incidence of HCC in patients with HBV-related liver cirrhosis is estimated around 3.7 persons/year^[12]. HCC may develop, but with a lower frequency also in patients with chronic HBV infection without cirrhosis, depending on demographic (male sex, older age), viral (higher levels of HBV replication; co-

Table 1 miRNAs involved in hepatitis B virus infection

miRNA	Validated target	Effect on HBV	Ref.
miRNA targeting HBV transcripts			
miR-15a/miR-16-1	HBx	↓	[28,33]
miR-20a/miR-92a-1	Polymerase/HBx	↓	[29]
miR-122	Polymerase/HBc	↓	[27]
miR-125a	HBsAg	↓	[23-26,32]
miR-199a-3p	HBsAg	↓	[22]
miR-205	HBx	↓	[31]
miR-210	HBsAg pre-S1 region	↓	[22]
miR-1231	HBc	↓	[30]
miRNAs targeting HBV regulators			
miR-1	HDAC4	↑	[51-53]
miR-15b	HNF1	↑	[50]
miR-34a	CCL22	↓	[60]
miR-122	Cyclin G1	↑	[43-45]
	HO-1		[45,46]
miR-130a	PGC1 PPAR	↓	[41]
miR-141	PPAR	↓	[24,40]
miR-146a	STAT1	↑	[58]
miR-152	DNMT-1	↑	[54-57]
miR-155	C/EBP	↓	[35-38]
	SOCS1	↓	[39]
miR-370	NFIA	↑	[49]
miR-372/373	NFIB	↑	[48]
miR-501	HBXIP	↑	[42]
miR-548a	IFN λ -1	↑	[59]

MiRNAs are listed according to their increasing number name. ↓: Suppress HBV infection; ↑: Promote HBV infection; HBV: Hepatitis B virus.

infections) and environmental (alcohol abuse) factors^[20].

MIRNAS ASSOCIATED WITH HBV INFECTION

So far, there is no experimental evidence confirming the synthesis of miRNAs by HBV, though a computational analysis suggested one HBV pre-miRNA candidate^[21]; however, HBV is able to modulate different host miRNAs, particularly through the transcriptional transactivator HBx protein. Conversely, different cellular miRNAs can regulate HBV gene expression and replication by a direct binding to HBV transcripts or indirectly targeting host factors, which in turn modulate viral replication.

Cellular miRNAs directly targeting HBV transcripts

The first miRNAs found to bind viral transcripts and repress HBV gene expression and replication were miR-210, miR-199-3p and miR-125a-5p. They were identified by two different experimental approaches (Table 1). In one procedure, Zhang *et al.*^[22] systematically screened for cellular miRNAs affecting HBV replication by a loss-of-function approach: Antagomirs targeting 328 miRNAs were transfected into a HepG2.2.15 cell model supporting full HBV replication, and then HBV surface antigen (HBsAg) expression was measured. Among the six miRNAs whose antagomirs caused an increase in HBsAg expression, miR-199a-3p and miR-210 were predicted to bind the HBsAg coding region and the HBV pre-S1 region, respectively;

the direct effect of miRNAs on viral transcripts were further validated by GFP reporter assay^[22]. In a different approach, Potenza *et al.*^[23] first predicted the potential targets of human hepatic miRNAs in different HBV sequence subtypes. The most promising targets were then subjected to a validation test based on cultured hepatic cells and luciferase reporter genes, demonstrating that miR-125a-5p was able to bind viral sequences. In particular, miR-125a was shown to be able to interfere with the HBsAg expression, since the transfection of miR-125a mimic or inhibitor into PLC/PRF/5 cell line that secretes HBsAg induced a marked decrease or enhancement in the amount of secreted HBsAg, respectively^[23]. Two independent studies then confirmed the ability of miR-125a to inhibit HBsAg translation: in a screening of HBV replication-related miRNAs, a pri-miR-125a expression vector could repress HBsAg synthesis in HepG2 cells^[24]; again, miR-125a mimic and inhibitor transfection in HepG2.2.15 cells resulted in an increase or decrease in HBV replication, respectively. The expression analysis of a panel of 814 miRNAs revealed that iron or TGF- α treatments, which increased or decreased HBV replication, respectively, had opposite effects on the expression of miR-125a, supporting its ability to interfere with HBV replication^[25,26].

Later, another study reported an inhibitory effect of a cellular miRNA on HBV replication: Chen *et al.*^[27] focused on the liver-specific microRNA, miR-122, first demonstrating its inhibitory effect on HBV gene expression and replication in cultured cells and then

validating its target sequence located at the coding region of the mRNA for the viral polymerase and the 3' untranslated region of the mRNA for the core protein^[27]. In a similar approach, two other studies found that miR-15a and miR-16-1 (differing by only one base outside the seed region) target HBx transcript and miR-20a/miR-92a-1 (belonging to miR-17-92 polycistron) and may inhibit HBV replication by targeting the viral transcripts^[28,29].

More recently, miR-1231 has also been shown to suppress HBV replication by targeting the HBV core (HBc) protein. In HBV-transfected HepG2 cells, overexpression of hsa-miR-1231 resulted in the suppression of HBV replication by targeting the HBV core protein^[30]. Also miR-205 was found to target a viral transcript, in particular HBx mRNA^[31]. miR-125a, miR-205 and miR-15/miR-16-1 expression were found to be modulated by HBx protein, resulting in an upregulation for miR-125a and downregulation for the others^[31-33]. These regulatory feedback loops may have an impact on the development of liver disease progressing to HCC, given the crucial role of HBx in hepatocarcinogenesis^[34].

Cellular miRNAs targeting regulators of HBV infection

HBV transcripts are under the control of four promoters and two enhancers (enhancer I and II), interacting with cellular factors that regulate HBV gene expression. Thus, miRNA regulation of these factors results in the modulation of HBV transcription and replication. Some miRNAs suppress HBV replication by targeting positive regulators of HBV (Table 1). One example is miR-155, which suppresses HBV transcription and replication, given its ability to target CAAT enhancer-binding protein (C/EBP), which is a positive regulator of HBV transcription through its binding to HBV enhancer II, core promoter and S promoter^[35-38]. miR-155 suppresses HBV infection also by modulating the host immune system (see below)^[39]. Also miR-141 is able to suppress HBV replication, since it represses at both the transcriptional and translational levels the peroxisome proliferator-activated receptor alpha (PPAR α), a transactivator of HBV promoters, with a critical role in HBV replication^[24,40]. Similarly, miR-130a reduced HBV replication by targeting two major metabolic regulators PGC1 α and PPAR γ , both of which can potentially stimulate HBV replication^[41].

Other miRNAs promote HBV replication by targeting negative regulators of HBV activity or by enhancing a positive regulator. miR-501 promotes HBV replication by targeting HBXIP, a negative modulator of HBV replication, because of its binding to the transactivation domain of HBx protein^[42]. A positive effect on HBV replication has also been described for miR-122, in contrast with that reported above. In particular, miR-122 can promote HBV replication in two ways: It prevents cyclin G1 from interacting with p53, which has a suppressive effect on HBV replication, and it targets heme oxygenase-1 (HO-1), an anti-HBV enzyme^[43,44];

miR-122 targets heme oxygenase-1 (HO-1), whose anti-HBV activity has been shown in HBV-transfected hepatoma cells and in persistently HBV replicating transgenic mice. HO-1 acts by decreasing stability of HBV core protein, thus blocking refill of nuclear HBV covalently closed circular (ccc)DNA^[45,46]. According to these mechanisms, the liver-rich miR-122 may have a similar role in stimulating the replication and gene expression of the two hepatotropic viruses, HCV and HBV^[47]. microRNA-372/373 promote the expression of HBV by targeting the nuclear factor I/B (NFIB), a transcription factor able to reduce viral HBsAg and HBeAg protein levels and viral core-associated DNA levels, due to its binding to enhancer I and core promoter of HBV^[48]. A similar mechanism has been described for miR-370, which suppresses HBV transcription and replication by targeting nuclear factor IA (NFIA)^[49]. Also miR-15b is able to target a negative regulator of HBV Enhancer I, *i.e.*, hepatocyte nuclear factor 1 α (HNF1 α) mRNA, thus resulting in the transactivation of HBV Enhancer I, in turn causing the enhancement of HBV transcription and replication^[50].

Another two miRNAs have a role in HBV transcription and replication by modulating epigenetic modifications such as histone modification and methylation. In particular, miR-1, by targeting histone deacetylase 4 (HDAC4) changes the expression of different genes, including an upregulation of farnesoid X receptor α , which enhances HBV transcription and replication by binding to the HBV core promoter^[51-53]. miR-152 has been shown to target DNA methyltransferase (DNMT-1), eventually resulting in a reduced methylation of covalently closed circular DNA (cccDNA), with an impact on the replicative activity of HBV^[54,55]. Consistently, miR-152 expression was also shown to be downregulated in the livers of HBx transgenic mice and inversely correlated with DNMT1 expression in HBV-related HCC patients^[56,57].

Some miRNAs may also have an indirect effect on HBV infection because of their role in the modulation of the host immune system. From the point of view of HBV, they represent a strategy to suppress antiviral immune responses, thus facilitating viral replication. This is the case of miR-146a and miR-548a, which promote HBV infection by suppressing T cell function through targeting Stat1 and by binding the 3'UTR of IFN- λ 1, respectively^[58,59]. Conversely, miR-34a and miR-155 suppress HBV infection by inhibiting Treg cell recruitment *via* chemokine CCL22 and augmenting the IFN signaling pathway, respectively^[39,60].

Overall, it is clear that the host-virus interaction in HBV infection is mediated not only by immune responses but also by miRNAs; during the co-evolution and adaptation between HBV and humans, complex miRNA-based networks have been established; this complexity may partly explain the opposing effects on HBV transcription and replication reported for some miRNAs (*e.g.*, miR-122).

MICRORNA DYSREGULATION IN HBV-RELATED HEPATOCELLULAR CARCINOMA

An aberrant expression of miRNAs has a causative effect on several pathological conditions, including cancer^[5]. In this field, several studies indicate that miRNAs can act as either tumor suppressors by downregulating the expression of oncogenes, or tumor promoters (oncomirs) by limiting the expression of oncosuppressor proteins^[61-63]. Cancer cell downregulation of Dicer, an enzyme playing a critical role in the biosynthesis of miRNAs, or mutations in its structure suppress miRNA biogenesis, leading to increased tumor progression^[64-67]. This implies that the oncosuppressive effect of miRNAs generally overcomes their oncogenic potential. It is also known that cell differentiation is often accompanied by increased Dicer expression^[68-70].

In 2011, Hou *et al.*^[71] performed an extensive study of the miRNomes of healthy human liver and HCC. In this paragraph, their work will be discussed in some detail since it provides a good example of the experimental procedures currently employed to study the miRNAs with oncogenic or oncosuppressive roles. The authors used a next-generation sequencing (NGS) technique to analyze human liver miRNome and found that 9 miRNAs accounted for about 90% of the liver miRNA content, with miR-122 being the most represented (52%). Other highly expressed miRNAs included miR-192 (16.9%), miR-199a/b-3p (4.9%), miR-101 (3.7%), let-7a (3.3%), miR-99a (2.2%), let-7c (2.1%), let-7b (1.7%), and let-7f (1.5%). MicroRNAs -199a-3p and -199b-3p have an identical nucleotide sequence but are transcribed from three genes, a-1, a-2, and b, with a2 being the most expressed in the liver. The authors then analyzed liver biopsies from patients with hepatocellular carcinoma by comparing tumor samples with adjacent non-cancer tissues. In HBV-related HCCs, a remarkable decrease in 199a-3p was observed. This result was then validated by qRT-PCR in a cohort of 40 HBV-related HCCs. This analysis showed that the miRNA was downregulated in 100% of the patients, with a mean decrease of 8.3-fold. Other miRNAs markedly downregulated were miR-99a and miR-125b, both decreased by about 7-fold in the majority of the patients. MicroRNA-122 and miR-125a were also downregulated (Table 2). On the other hand, miR-21 was upregulated by 4.8-fold in half of the tumor samples. In the same study, genomic analyses of DNA samples from liver biopsies indicated that miR-199a-3p downregulation was not due to gene deletion or promoter DNA methylation but to histone modification and subsequent repression of transcription. Biological assays were then performed showing that transfection of a synthetic miR-199a-3p mimic in cultured HCC cell lines repressed cell proliferation and induced apoptosis, thus suggesting a tumor suppressive role *in vitro*. Experiments *in vivo* were then performed by monitoring

human HCC cell growth in nude mice. Intra-tumoral injection of cholesterol-conjugated miR-199a-3p or its over-expression with a recombinant adeno-associated virus system markedly decreased tumor growth, further supporting its tumor suppressive role. The mechanism of action of miR-199a-3p was then studied. A computational analysis with TargetScan was used to identify the human genes whose mRNA sequence may allow binding of miR-199a-3p, possibly leading to gene silencing. Most of them were associated with the mitogen-activated protein kinase (MAPK) pathway, thus providing a possible explanation for the anti-proliferative activity of the miRNA. Among them, PAK4 was downregulated by miR-199a-3p transfection in HCC cells, and this effect was found to be due to a direct interaction with the gene transcript using a luciferase-based reporter assay. Finally, measurement of the miR-199a-3p content in the liver biopsies from two other cohorts of 142 and 152 patients showed that a markedly decreased HCC level of the miRNA correlated with poor survival. It should be noted that a downregulation of miR-199a-3p in HCC had already been observed in 2006 by Murakami *et al.*^[72] employing much less powerful miRNA detection techniques based on microarrays and Northern blotting analyses. The same study had also shown a tumor downregulation of miR-125a. Since then, other studies have confirmed the tumor-suppressive role of miR-199a-3p in HCC and identified CD44, CD51, c-MET, mTOR, YAP1, and ZHX1 as other direct miRNA targets contributing to its anti-proliferative and pro-apoptotic effects^[73-78] (Table 2). The ability of miR-199a-3p to downregulate several target proteins should not surprise given the pleiotropic effects of microRNAs that are able to interact with several mRNAs provided with the same or similar binding sites located in their 3'-UTR. Therefore, a single microRNA often silences several genes with related functions, thus showing a marked effect on the cell physiology.

Other studies conducted with similar experimental approaches, profiling microRNAs by qPCR-arrays on multi-well plates, have confirmed the HCC downregulation of miR-99a, -122, -125a, -125b, and the upregulation of miR-21, also identifying their molecular targets (Table 2). Other miRNAs consistently downregulated in HCC included the let-7 family of microRNAs and miR-29; other examples of upregulated miRNAs were miR-155 and -221 (Table 2). These data have raised substantial interest in microRNAs in the field of molecular and cellular oncology, leading to the publication of several interesting reviews^[79-84].

As regards the reasons for deregulated miRNA expression in HCC, it should be noted that the viral HBx protein plays a primary role in HBV cancerogenesis^[85]. It is a transcriptional trans-activator lacking a DNA-binding domain but able to interact with several transcription factors^[86,87]. Therefore, it is not surprising that HBx can modify the hepatic miRNA expression^[88]. It is noteworthy that HBx downregulates the expression of

Table 2 MicroRNAs playing oncogenic or oncosuppressive roles in hepatocellular carcinoma

MicroRNA	Expression in HCC	Cellular effects	Direct targets in HCC	Ref.
let-7 family	Downregulated	Proliferation (-) migration (-) apoptosis (+)	Bcl-xL, c-Myc, collagen type 1 α 2, RAS, STAT3	[89,132-136]
miR-21	Upregulated	Proliferation (+) migration (+) apoptosis (-)	IL-12, HBP1, PDCD4, PTEN, RECK, TIMP-3	[72,137-141]
miR-29	Downregulated	Proliferation (-) apoptosis (+)	Bcl-2, lncRNA MEG3, Mcl-1, SIRT1	[80,142,143]
miR-99a	Downregulated	Proliferation (-)	AGO2, IGF1R, mTOR	[144-147]
miR-122	Downregulated	Proliferation (-) migration (-)	ADAM17, c-Myc, CUTL1, CCNG1, WNT1	[145,148-153]
miR-125a	Downregulated	Proliferation (-) migration (-) angiogenesis (-)	c-RAF, LIN28B, MMP11, SIRT7, VEGFA, Zbtb7a	[72,123,145,154-159]
miR-125b	Downregulated	Proliferation (-) apoptosis (+)	Bcl-2, LIN28B, SIRT7	[72,145,157,160-162]
miR-155	Upregulated	Proliferation (+) migration (+)	ARID2, C/EBPbeta, PTEN, SOCS1, SOX6	[38,163-165]
miR-199a-3p	Downregulated	Proliferation (-) apoptosis (+)	CD44, CD51, c-MET, mTOR, PAK4, YAP1, ZHX1	[71-78,154]
miR-221	Upregulated	Proliferation (+) apoptosis (-)	BMF, Caspase-3, CDKN1B, CDKN1C, DDIT4	[166-169]

ADAM17: Disintegrin and metalloprotease 17; AGO2: Argonaute-2 protein; Bcl-2: B-cell lymphoma 2 protein; ARID2: AT-rich interactive domain 2; BMF: Bcl-2-modifying factor; C/EBPbeta: CCAT/enhancer binding protein beta; CCNG1: Cyclin-G1; CDKN: Cyclin-dependent kinase inhibitor; DDIT4: DNA-damage inducible transcript 4; HBP1: HMG-box transcription factor 1; IGF1R: Insulin-like growth factor 1 receptor; IL-12: Interleukin-12; Mcl-1: Induced myeloid leukemia cell differentiation protein 1; PAK4: Serine/threonine-protein kinase 4; PDCD4: Programmed cell death protein 4; PTEN: Phosphatase and tensin homolog; RECK: Reversion-inducing-cysteine-rich protein with kazal motifs; SIRT: Sirtuin; SOCS1: Suppressor of cytokine signaling 1; STAT3: Signal transducer and activator of transcription 3; TIMP3: Metalloproteinase inhibitor 3; VEGFA: Vascular endothelial growth factor A; YAP1: Yes-associated protein 1; Zbtb7a: Zinc finger and BTB domain-containing protein 7A; ZHX1: Zinc-fingers and homeoboxes-1.

oncosuppressive miRNAs let-7 and miR-122, whereas it upregulates oncomirs -21 and -221. MicroRNA-125a is induced by HBx^[32,89] but is downregulated by its carboxyl-terminal truncated variant that is frequently found in HBV-related HCC^[90]. These data indicate that the tumorigenic effect of HBx is partially mediated by microRNAs. Besides HBx, dysregulation of microRNAs in cancer cells may be determined by other genetic or epigenetic factors^[91]. Chromosomal abnormalities, deletions, and mutations can downregulate cellular miRNA expression^[92,93], and several miRNA genes associated with CpG islands are also transcriptionally repressed by promoter DNA methylation^[94].

MICRORNAS AS BIOMARKERS OF HBV-RELATED LIVER DISEASES

Biomarkers of liver damage and treatment response in chronic hepatitis B

As mentioned above, chronic HBV infection is associated with a wide spectrum of clinical manifestations: An inactive carrier state, chronic hepatitis of different grade of activity and liver cirrhosis in different stages of compensation, with or without HCC. The mechanisms of virus/host interactions leading to different outcomes have been only partially clarified and a substantial contribution to their knowledge is expected from the studies on the expression profile of microRNAs and their role in liver fibrogenesis. To this regard, one of the most interesting microRNAs is miR-122, which

accounts for 50%-70% of all miRNAs expressed in the human liver. Several investigations^[90-97] have shown a higher miR-122 serum concentration in patients with chronic HBV infection than in normal subjects and a correlation between its serum levels and HBV load, HBsAg titers and liver biochemistry. However, the interpretation of these data as a consequence of an upregulation of microRNA requires the exclusion of the possibility that they could be a consequence of an increased release of miR-122 in the blood due to concomitant hepatic cytolysis. To this regard, Wang *et al.*^[44] showed that the miR-122 expression in the liver was significantly downregulated in 41 Chinese patients with HBV infection compared with 10 healthy controls, and that the miR-122 levels negatively correlated with the intrahepatic viral load and with the degree of necroinflammation, confirming *in vivo* the inhibitory activity of miR-122 on HBV replication.

Interesting information on the correlation between microRNAs and HBV-related liver damage comes from some investigations on miR-29. A study^[98] performed on serum samples of 91 HBV-infected patients and 12 healthy controls demonstrated a downregulation of this miRNA in patients with more advanced liver fibrosis. In addition, the serum levels of three microRNAs (miR-29a, miR-143, miR-223) predicted the progression of liver fibrosis better than APRI or FIB-4 tests in 123 Chinese patients with chronic HBV infection^[99].

In 2013, we identified the miR-125a-5p as an independent predictor of more severe liver lesions (necroinflammation and fibrosis) in a cohort of 27 treat-

ment-naïve patients with HBeAg-negative chronic hepatitis B^[100], findings confirmed by the data of a study by Zheng *et al.*^[101] on 91 HBV-infected patients. More recently, a Chinese study performed on 211 patients with chronic hepatitis B demonstrated that serum concentrations of miR-125b, a microRNA classified in the same family, correlate with the histological activity and HBV load^[102].

The clinical use of microRNAs in chronic HBV infection may go beyond the assessment of liver damage. For example, Brunetto *et al.*^[103] reported that the use of a serum six miRNAs signature (MiR-B-Index) correctly discriminated 61 HBV subjects in a naturally inactive stage and 84 in a stage of treatment-induced immune-control. More recently, pre-treatment serum levels of two microRNAs predicted the off-treatment biochemical and virological response after a 48-wk combination therapy with Peg-IFN and adefovir, the miR-301a-3p in 41 HBeAg-positive patients and the miR-145-5p in 45 HBeAg-negative patients^[104].

Biomarkers and therapeutic targets in HBV-related HCC

HCC is the fifth most common cancer in men and the ninth in women, with respectively 554000 and 228000 new cases per year worldwide^[105]. In addition, HCC is the second most common cause of death for cancer worldwide, responsible for nearly 750000 deaths per year, half of which in HBV-infected patients. Despite the great efforts of the scientific communities and Healthcare Authorities, the mortality rate of HCC has not significantly decreased in the last decade, mainly because the diagnosis is very late in most cases. In fact, the level of serum alpha-fetoprotein (α -FP) lacks sensitivity and is no longer indicated for screening^[106,107] and the imaging diagnostic techniques require quality of equipment and considerable experience by the radiologists. In addition, the use of sorafenib, the only treatment shown to improve the overall survival of patients in the advanced stages^[108] is limited by the high rates of adverse reactions and treatment failures^[109].

In this context, many microRNAs have been found dysregulated in serum and liver of HCC patients and therefore considered as possible diagnostic biomarkers and therapeutic targets^[84,110-113]. In 2011, Zhou *et al.*^[111] screened for 723 microRNAs the serum samples of 934 Chinese patients with HBV-related chronic hepatitis, cirrhosis or HCC and identified and validated a panel of 7 miRNAs providing a high diagnostic accuracy for HCC, regardless of cancer stage. Subsequently, the serum level of miR-21 was proposed as a novel biomarker of HCC^[112]. The diagnostic accuracy of miR-21 serum level has been further investigated in several subsequent studies^[113-115] and in a meta-analysis^[116] including 677 patients of different etiologies, with 81.2% sensitivity and 84.8% specificity in the diagnosis of HCC.

In 2010, Li *et al.*^[117] identified a panel of 13 miRNAs differentially present in serum samples of 120 HBV-related HCC, 135 HBV-infected patients and 210

healthy controls. Using a panel with miR-25, miR-375, and let-7f, they obtained a 97.9% sensitivity and 99.1% specificity in HCC prediction. Furthermore, the miR-375 proved to be of high diagnostic accuracy in two prospective Chinese cohorts^[118].

The tissue expression of several miRNAs in HCC tissue has been investigated to identify therapeutic targets. Gao *et al.*^[119] analyzed the expression profile of 7 miRNAs in 24 dysplastic nodules, 29 HCC tissues and 40 non-tumoral liver tissues surrounding HCC from HBV-infected patients and found a downregulation of miR-145 and miR-199b and an upregulation of miR-224. They also demonstrated that the restoration of miR-145 in both HepG2 and Hep3B HCC cells significantly inhibited cell proliferation and reduced cell migration and invasion. As mentioned above, miR-122 is downregulated in liver tissue of patients with chronic hepatitis B, and its concentration is inversely correlated with the degree of liver fibrosis. This downregulation was reported also in 19 HBV-related HCC tissues by Li *et al.*^[120] in 2013; they also demonstrated that the pituitary tumor-transforming gene 1 (PTTG1) binding factor (PBF), a validated molecular target of miR-122, enhances the proliferation and invasion of HCC cells, while its silencing induced a significant reduction in tumor growth in a murine HCC model. It has also been demonstrated that the deletion of mouse Mir122 resulted in hepatosteatosis, hepatitis, and the development of tumors resembling HCC^[121], while its re-expression reduced disease manifestations and tumor incidence^[122]. We recently reported a lower expression of miR-125a-5p in HCC tissues compared with non-tumor tissue in 55 patients with hepatocellular cancer of different etiologies^[123]. In addition, we found a significant upregulation of three oncogenes in HCC tissue, MMP-11, c-Raf and Sirt-7, already validated as molecular targets of miR-125a-5p, an observation that provides an explanation for the tumor suppressor activity exerted by this microRNA.

FUTURE PERSPECTIVES: RNA-INTERFERENCE IN THE TREATMENT OF CHRONIC HEPATITIS B AND HEPATOCELLULAR CARCINOMA

Some literature data suggest that microRNA-interference might be useful in the treatment of HBV-related chronic hepatitis and HCC. The RNA-interference directed to inhibit HBV replication has been investigated in several animal models and, more recently, in a clinical study^[124]. In a phase 2 clinical trial enrolling entecavir-naïve or -exposed HBsAg-positive patients with chronic hepatitis^[125], ARC-520, a mixture of small-interfering RNAs (siRNAs) targeting all viral transcripts, induced HBsAg reduction up to 1.5 log10 in HBeAg-positive and up to 0.5 log10 in HBeAg-negative subjects with a single intravenous administration of up to 4 mg/kg. The reasons for the limited efficacy in HBeAg-

negative patients have been more recently investigated in a preclinical study on chimpanzees^[126]. The authors demonstrated a lack of target sites for the siRNAs in the HBV DNA integrated in the host genome, which represents the dominant source of viral transcripts in HBeAg-negative patients. These findings highlight a novel issue that should be addressed by future research on HBV treatment. Other two RNAi-based therapies (TKM-HBV and ALN-HBV) are currently investigated in chimpanzees and mice with promising preliminary results^[127].

There is some evidence of the efficacy of miRNAs mimics or inhibitors both in preclinical studies and in a recent phase I clinical trial regarding the treatment of hepatocellular cancer. In an HCC murine model, the systemic administration of miR-26a using an adeno-associated virus resulted in an inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and protection from disease progression^[127-129]; a cholesterol-modified isoform of anti-miR-221 can reduce tumor cell proliferation and increase the tumor doubling time and the survival in mice with hepatocellular cancer. An miR-375 mimic delivered in gold nanoparticles has shown therapeutic efficacy without significant toxicity in primary and xenograft tumor mouse models^[130]. Finally, MRX34, a liposomal miR-34a mimic, showed anti-tumor activity in a phase I clinical trial enrolling patients with refractory advanced primary liver cancers or other solid neoplasms^[131], but this trial has been stopped because of serious adverse events.

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Treatment strategies for advanced hepatocellular carcinoma: Sorafenib *vs* hepatic arterial infusion chemotherapy

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Abstract

Sorafenib is used worldwide as a first-line standard

systemic agent for advanced hepatocellular carcinoma (HCC) on the basis of the results of two large-scale Phase III trials. Conversely, hepatic arterial infusion chemotherapy (HAIC) is one of the most recommended treatments in Japan. Although there have been no randomized controlled trials comparing sorafenib with HAIC, several retrospective analyses have shown no significant differences in survival between the two therapies. Outcomes are favorable for HCC patients exhibiting macroscopic vascular invasion when treated with HAIC rather than sorafenib, whereas in HCC patients exhibiting extrahepatic spread or resistance to transcatheter arterial chemoembolization, good outcomes are achieved by treatment with sorafenib rather than HAIC. Additionally, sorafenib is generally used to treat patients with Child-Pugh A, while HAIC is indicated for those with either Child-Pugh A or B. Based on these findings, we reviewed treatment strategies for advanced HCC. We propose that sorafenib might be used as a first-line treatment for advanced HCC patients without macroscopic vascular invasion or Child-Pugh A, while HAIC is recommended for those with macroscopic vascular invasion or Child-Pugh A or B. Additional research is required to determine the best second-line treatment for HAIC non-responders with Child-Pugh B through future clinical trials.

Key words: Treatment strategy; Hepatic arterial infusion chemotherapy; Sorafenib; Hepatocellular carcinoma

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Core tip: In Japan, sorafenib and hepatic arterial infusion chemotherapy (HAIC) are described as treatment options for hepatocellular carcinoma (HCC). Although no randomized controlled trials have compared these treatments, retrospective analyses have shown similar survival between them. Sorafenib is generally used for Child-Pugh A, while HAIC is indicated for Child-Pugh A or B. Compared to sorafenib, HAIC shows better responses in cases exhibiting macroscopic vascular invasion. After reviewing treatment strategies for advanced HCC, we recommended sorafenib as first-line treatment for cases without macroscopic vascular invasion or Child-Pugh A, and HAIC for those with macroscopic vascular invasion or Child-Pugh A or B.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most

common cause of cancer related death worldwide^[1]. According to the Global Burden of Disease 2015 study of 195 countries, the number of liver cancer cases increased by 75% between 1990 and 2015, and hepatitis B virus (HBV) was responsible for 33% of global liver cancer mortality compared to 30% from alcohol, 21% from hepatitis C virus (HCV), and 15% from other causes^[2]. However, the incidence of both HBs antigen-negative and HCV antibody-negative HCC (non-B, non-C HCC) has recently increased in Japan^[3,4]. As there are not yet any established surveillance programs for non-B, non-C HCC patients, it is difficult to diagnose such patients at an earlier disease stage. Therefore, the number of advanced HCC patients at the time of diagnosis may be increasing in Japan. Additionally, even if an earlier disease stage of HCC is detected, many patients progress to an advanced stage because of frequent recurrence of the disease. Therefore, it is now more important than ever to develop a treatment for advanced HCC. In this review, we review the treatment strategies for advanced HCC, particularly sorafenib and hepatic arterial infusion chemotherapy (HAIC).

GUIDELINES FOR ADVANCED HCC

The results of the global investigation of therapeutic decisions in HCC and of its treatment with sorafenib (GIDEON) study show differences in the management of HCC, including diagnosis, treatment, and monitoring, among several regions. In consequence, there have been regional differences in patient outcomes^[5]. Although several guidelines for the clinical management of HCC have been established worldwide, there are some differences in the treatment algorithms among these guidelines. Table 1 shows the major recent guidelines from Asia, Europe and the United States^[6-13]. The Barcelona clinic liver cancer (BCLC) staging system, which stratifies patients by tumor stage and underlying liver disease, is widely accepted in clinical practice^[14]. Among the five HCC stages (BCLC 0, A, B, C and D), the advanced BCLC C stage includes symptomatic patients with performance status (PS) 1-2, vascular invasion, extrahepatic spread, or a combination thereof^[14]. For patients with BCLC C and good liver function (Child-Pugh A), sorafenib is the preferred first-line treatment according to guidelines from Europe and the United States^[11-13]. According to guidelines from Asia^[7-9], systemic therapy (molecular-targeted drugs) or transcatheter arterial chemoembolization (TACE) is recommended as standard treatment for such patients. However, HAIC is not generally recommended as a standard of care in the above-mentioned guidelines.

Whereas sorafenib and HAIC are indicated for the patients with minor portal vein invasion (so-called Vp1, 2) or portal invasion at the first portal branch (so-called Vp3) in the Japan Society of Hepatology and Liver Cancer Study Group of Japan (JSH-LCSGJ) Consensus-based Treatment Algorithm for HCC revised in 2014, HAIC, but not sorafenib, is recommended for portal invasion at the main trunk of the portal vein (so-called Vp4)^[6]. Fur-

Table 1 Guidelines for the clinical management of hepatocellular carcinoma

	Publishing year	Guidelines	Drafted by	Treatment algorithm for advanced HCC (BCLC C)	Ref.
Asia	2014	JSH-LCSGJ	Japan Society of Hepatology and Liver Cancer Study Group of Japan	HAIC (Vp1-4), Sorafenib (Vp1-3), TACE (Vp1, 2), Resection (Vp1, 2)	[6]
	2014	KLCSG-NCC	Korean Liver Cancer Study Group and National Cancer Center	TACE, Sorafenib	[7]
	2014	HKLC	Hong Kong Liver Cancer	Systemic therapy, Supportive care	[8]
	2017	APASL	Asian-Pacific Association for the Study of the Liver	Systemic therapy (sorafenib and regorafenib), TACE for patients with no extrahepatic metastasis	[9]
	2017	JSH	Japan Society of Hepatology	TACE, Resection, HAIC, Molecular targeted agents	[10]
Europe	2018	EASL	European Association for the Study of the Liver	Sorafenib (sorafenib, lenvatinib, regorafenib, and cabozantinib)	[11]
	2012	ESMO-ESDO	European Society for Medical Oncology and European Society of Digestive Oncology	Sorafenib	[12]
United States	2011	AASLD	American Association for the Study of Liver Disease	Sorafenib	[13]

HAIC: Hepatic arterial infusion chemotherapy; Vp1-4: Portal vein invasion according to JSH-LCSGJ; TACE: Transcatheter arterial chemoembolization; BCLC: Barcelona clinic liver cancer.

thermore, according to the most recent version (2017) of the Clinical Practice Guidelines for HCC proposed by JSH, TACE, resection, HAIC, and molecular-targeted agents are equally recommended for HCC patients with portal invasion. It has also been argued that the treatment should be selected after considering all of the patient's conditions as a whole^[10].

Finally, the 2017 version of the National Comprehensive Cancer Network (NCCN) Guidelines supports HAIC for unresectable HCC; however, its use in the context of a clinical trial is preferred^[15].

SORAFENIB FOR ADVANCED HCC

Current status of sorafenib

Sorafenib is an oral multi-targeted kinase inhibitor that suppresses tumor growth, and it was the first drug to demonstrate a survival benefit in patients with advanced HCC. In two large-scale Phase III trials, although the response rate of sorafenib was only 2%-3.3% according to the Response Evaluation Criteria in Solid Tumors (RECIST), sorafenib treatment significantly improved overall survival (OS) [sorafenib vs placebo median survival time (MST): 10.7 mo vs 7.9 mo, hazard ratio (HR): 0.69, $P < 0.001$ in the SHARP trial; and MST: 6.5 mo vs 4.2 mo, HR: 0.68, $P = 0.014$ in the Asia-Pacific trial] and the time-to-progression (TTP) (sorafenib vs placebo median TTP: 5.5 mo vs 2.8 mo, HR: 0.58, $P < 0.001$ in the SHARP trial; and TTP: 2.8 mo vs 1.4 mo, HR: 0.57, $P = 0.0005$ in the Asia-Pacific trial) in patients with advanced HCC^[16,17]. Therefore, sorafenib is utilized as a standard first line agent for the treatment of advanced HCC worldwide^[6-13]. Recently, Rimola *et al.*^[18] reported that 1% of patients treated with sorafenib (12/1119) exhibited complete response (CR), according to RECIST, and the MST for those patients was 85.8 mo.

For several years, antiangiogenic tyrosine-kinase

inhibitors other than sorafenib have failed in Phase III clinical trials^[19,20]. However, recent studies have demonstrated the efficacy of two oral multi-kinase inhibitors, the second-line agent regorafenib, which is used for sorafenib-resistant HCC, and the first-line agent lenvatinib, which has been shown to be non-inferior to sorafenib for OS^[21,22].

Regorafenib has been reported as a second-line agent following sorafenib because of improvement in OS (regorafenib vs placebo MST: 10.6 mo vs 7.8 mo, HR: 0.63, $P < 0.0001$) (RESORCE trial)^[21]. According to the results of this study, regorafenib was approved in the United States and Japan in 2017.

Lenvatinib is an oral multi-target inhibitor of vascular endothelial growth factor (VEGF) receptors 1-3, fibroblast growth factor receptors 1-4, platelet-derived growth factor receptor alpha, KIT, and RET^[23]. A comparative global Phase III trial of lenvatinib in the first-line setting (REFLECT trial) demonstrated non-inferiority to sorafenib in advanced HCC patients (lenvatinib vs sorafenib MST: 13.6 mo vs 12.3 mo, HR: 0.92)^[22]. In addition, the progression-free survival (PFS), TTP, and overall response rate (ORR) were significantly better in patients treated with lenvatinib than in those treated with sorafenib (lenvatinib vs sorafenib, median PFS: 7.4 mo vs 3.7 mo, HR: 0.66, $P < 0.0001$; median TTP: 8.9 mo vs 3.7 mo, HR 0.63, $P < 0.0001$; ORR: 24.1% vs 9.2%, $P < 0.0001$). Lenvatinib is approved for unresectable thyroid cancer and has been usable for HCC in Japan prior to it being approved in the rest of the world. However, HCC patients with 50% or higher liver occupation, bile duct invasion, or main portal invasion met the exclusion criteria of the REFLECT trial. Such HCC patients may be candidates for general usage of sorafenib.

Predictive factors for response and survival

Bruix *et al.*^[24] conducted analyses of two large trials

(827 patients, SHARP and Asia-Pacific trials) and reported prognostic factors. According to this report, vascular invasion, high alpha-fetoprotein (AFP), and high neutrophil-lymphocyte ratio (NLR) were prognostic factors for poorer OS, while lack of extrahepatic spread, HCV, and low NLR were predictive factors for greater sorafenib benefit^[24]. Among serum and plasma factors, VEGF^[25-27], angiopoietin-2 (Ang-2)^[25,26], AFP^[25,26,28-31], NLR^[32,33], TIE-2 expressing monocytes (TEMs)^[34], microRNA^[35-37], and circulating tumor cells (CTCs)^[38] have been identified as potential biomarkers (Table 2). The expression of phospho-ERK^[39-41], phospho-c-Jun^[42], and VEGFR-2^[41], and amplification of FGF3/FGF4^[43], have been identified as possible predictive biomarkers in tissues (Table 3). In studies of imaging biomarkers, it has been reported that decreased blood flow after sorafenib treatment^[44] and low pretreatment standardized uptake values of ¹³F-fluorodeoxyglucose (FDG) in positron emission tomography (PET)^[45] are associated with prolonged OS. Although there have been several reports of a correlation between adverse effects (hypertension, skin toxicity, diarrhea, etc.) and sorafenib efficacy, it has been difficult to establish conclusions because of difference in the frequencies of these adverse effects among patients of different races. However, Howell *et al.*^[46] reported that patients with sorafenib-related toxicity such as diarrhea, hypertension, and hand-foot syndrome, had good prognoses in a large, multicenter prospective cohort study. Furthermore, the potential of other biomarkers has been explored^[47]. Although several studies have investigated predictive biomarkers for response and survival associated with sorafenib, no such biomarkers have been established.

HAIC FOR ADVANCED HCC

Current status of HAIC

In HAIC, as it is theoretically possible to accumulate local concentrations of anti-cancer drugs in the liver and to reduce their systemic distribution, it is believed to have a stronger antitumor effect and lower incidence of adverse reactions compared with systemic chemotherapy. On the other hand, one disadvantage is the need to master the HAIC procedure, and several adverse effects are associated with HAIC including inflammation of blood vessels, arterial obstructions, peptic ulcers due to drug leakage, and infections or obstructions of reservoir catheters.

According to the 2017 version of the treatment algorithm for HCC produced by JSH^[10], HAIC is recommended as a second-line treatment for patients with ≥ 4 HCCs and an absence of portal invasion, while HAIC is considered a first-line treatment for those with portal invasion.

HAIC has become widely used in Asia, especially Japan, where the main HAIC regimens are low-dose cisplatin (CDDP) combined with 5-fluorouracil (5-FU) (low-dose FP)^[48-51], interferon (IFN) in combination with

5-FU (FAIT)^[50,52,53], and CDDP alone^[51,54-56] (Table 4). In both low-dose FP and FAIT regimens, the key drug is 5-FU. In addition, CDDP or IFN exert their own effects to amplify the effect of 5-FU, and they are therefore considered biochemical modulators of 5-FU. Moreover, one benefit of the CDDP alone regimen is that a catheter is inserted each time, making the troublesome implantation of a reservoir catheter unnecessary. The regimens using low-dose FP or FAIT have response rates of approximately 30%-40%, while the CDDP alone regimen has rates of approximately 20%-30% (Table 4)^[48-53,55-57]. Survival is significantly better in patients with radiological response [CR or partial response (PR)] (so-called responders) than in patients with radiological no-response (stable or progressive disease) (so-called non-responders).

The principal reasons for low clinical recognition of HAIC are the small sample size of almost all studies and the lack of large randomized trials. However, effective results have been demonstrated by previous studies. In a report comparing the FAIT regimen of HAIC with historical controls, HAIC was shown to significantly improve survival^[53]. A Japanese nationwide survey supported the efficacy of the low-dose FP regimen of HAIC for treating advanced HCC^[49]. After adjusting for known risk factors, survival benefits of this therapy were evident (HR: 0.48, 95%CI: 0.41-0.56, $P < 0.0001$). In a propensity score-matched analysis, the MST was longer in patients who received HAIC ($n = 341$, 14.0 mo) than in those who did not receive active treatment ($n = 341$, 5.2 mo) (HR: 0.60, 95%CI: 0.49-0.73, $P < 0.0001$). In cases of Child-Pugh A or B disease with more than three tumors (370 propensity score-matched patients), the MST was longer in patients treated with HAIC (13.9 mo) than in those with no therapy (3.7 mo) ($P < 0.0001$). In cases of Child-Pugh A or B disease with portal vein tumor thrombus (378 propensity score-matched patients), the MST was also longer in patients treated with HAIC (7.9 mo) than in those with no therapy (3.1 mo) ($P < 0.0001$).

Predictive factors for response and survival

As HAIC is selected for advanced HCC patients with poor prognoses, it is important to identify predictive factors for response and survival (Table 5)^[48,49,53,58-61].

The predictive factors for poor response to HAIC include the presence of vascular invasion^[58], the presence of extrahepatic metastasis^[58], $\text{NLR} \geq 2.87$ ^[58], a concentration of serum VEGF ≥ 100 pg/mL^[60], a negative HCV antibody test result^[61], and a platelet count $\geq 15 \times 10^4/\mu\text{L}$ ^[61], and a negative des-gamma-carboxy prothrombin (DCP) response [defined as a reduction of $< 20\%$ or an increase from baseline after a half course of HAIC (2 wk)]^[48].

Survival benefits for HAIC have been reported in HAIC responders^[53,60,61]. However, therapeutic effect is not an effective prognostic predictor. The poor prognostic predictors include not only tumor-associated factors,

Table 2 Serum and plasma biomarkers of sorafenib response and survival

Biomarkers	Ref.	Publishing year	Case number	Predictive factors for response	Predictive factors for survival	Others
VEGF	Llovet <i>et al</i> ^[25]	2012	299	No predictive value	Not prognostic value	
	Miyahara <i>et al</i> ^[26]	2013	120	No predictive value	Not prognostic value	
	Tsuchiya <i>et al</i> ^[27]	2014	63	No predictive value	VEGF response (a > 5% decrease during 8 wk of treatment): Better OS	
Ang-2	Llovet <i>et al</i> ^[25]	2012	299	No predictive value	Low Ang-2: Better OS	
	Miyahara <i>et al</i> ^[26]	2013	120	High Ang2: PD	Low Ang-2: Better OS	
Changes of AFP	Personeni <i>et al</i> ^[28]	2012	85	AFP response (a > 20% decrease during 8 wk of treatment): Better ORR, DCR	AFP response: Better OS	
	Yau <i>et al</i> ^[29]	2011	94	AFP response (a > 20% decrease during 6 wk of treatment): Better DCR	AFP response: Better PFS	
	Kuzuya <i>et al</i> ^[30]	2015	47	-	High AFP ratio (a > 1.2 at 2 wk relative to baseline): Poor OS	High poor prognostic score (the absence of disappearance of arterial tumor enhancement on CE-CT, AFP ratio of > 1.2, and two or more increments in CP score after 2 wk of Treatment): Poor OS and DCR
	Nakazawa <i>et al</i> ^[31]	2013	59	AFP increase (more than 20% from baseline during 4 wk of treatment): PD	AFP increase: Better OS and PFS	
AFP	Llovet <i>et al</i> ^[25]	2012	299	-	AFP > 200 ng/mL: Poor OS	
	Miyahara <i>et al</i> ^[26]	2013	120	-	Not prognostic value	
	Kuzuya <i>et al</i> ^[30]	2015	47	-	Not prognostic value	
NLR	Zheng <i>et al</i> ^[32]	2013	65	-	High NLR (> 4): Poor OS and TTP	
	Howell <i>et al</i> ^[33]	2017	175	-	High NLR (> 2.52): Poor OS	
TEMs	Shoji <i>et al</i> ^[34]	2017	25	High ΔTEMs (changes in TEMs before and at 1 mo after therapy): PD	High ΔTEMs (changes in TEMs before and at 1 mo after therapy): Poor OS	
	Stiuso <i>et al</i> ^[35]	2015	39	Upregulation of miR-423-5p after treatment: SD or PR	-	
MicroRNA	Yoon <i>et al</i> ^[36]	2017	24	-	Low miR-10b-3p: Poor OS	
	Nishida <i>et al</i> ^[37]	2017	53	High miR-181a-5p: PR + SD	High miR-181a-5p: Better OS	
	Li <i>et al</i> ^[38]	2016	59	pERK+/pAkt- CTCs: Better DCR	pERK+/pAkt- CTCs: Better DCR	

Ang-2: Angiopoietin-2; CE-CT: Contrast-enhanced computed tomography; NLR: Neutrophil to lymphocyte ratio; AFP: Alpha-fetoprotein; CTC: Circulating tumor cells; TEMs: TIE-2-expression monocytes; VEGF: Vascular endothelial growth factor; PD: Progressive disease; OS: Overall survival; DCR: Disease control rate; ORR: Overall response rate; PFS: Progression-free survival; CP: Child-Pugh; pERK: Phosphorylated extracellular signal-regulated kinase; PR: Partial response; SD: Stable disease; TTP: Time to progression.

such as more than three tumors^[49], large tumors (> 3 cm)^[49], the presence of vascular invasion^[49,53], the presence of extrahepatic metastasis^[49,58,61] and high AFP levels^[49,58,61], but also those associated with the patient, including dysfunction of the liver reserve^[48,49,53,58-61], ECOG PS 1-2^[58,61], and a positive HBs antigen test result^[49]. Additionally, poor prognostic predictors include negative responses of AFP or DCP^[48], high levels of inflammation-related markers such as NLR and CRP^[58], low transferrin levels (< 190 mg/dL)^[59] and high VEGF levels (\geq 100 pg/mL)^[60].

A new assessment score: Assessment for continuous treatment with HAIC

It is important to identify the effective benefit of early HAIC treatment in HCC patients. Therefore, we developed a new therapeutic assessment score to guide decisions regarding HAIC treatment, the Assessment for Continuous Treatment with HAIC (ACTH)^[48]. The ACTH score (range, 0-3) is calculated from simple three parameters: Child-Pugh score before HAIC (A = 0, B = 1), AFP response (yes = 0, no = 1), and DCP response (yes = 0, no = 1). The tumor markers' responses are

Table 3 Tissue biomarkers of sorafenib response and survival

Biomarkers	Ref.	Publishing year	Case number	Predictive factors for response	Predictive factors for survival
Expression of p-ERK	Abou-Alfa <i>et al</i> ^[39]	2012	33	-	High pERK: Longer TTP
	Chen <i>et al</i> ^[40]	2013	54	-	High pERK: Longer TTP
	Negri <i>et al</i> ^[41]	2015	77	-	High pERK: Shorter OS and PFS
Expression of p-c-Jun	Hagiwara <i>et al</i> ^[42]	2012	39	High p-c-jun: Poor response	High p-c-jun: Shorter TTP and OS
Expression of VEGFR-2	Negri <i>et al</i> ^[41]	2015	54	-	High VEGFR-2: Shorter OS and PFS
FGF3/FGF4 amplification	Arao <i>et al</i> ^[43]	2013	48	FGF3/FGF4 amplification: Responder	-

ERK: Extracellular signal-regulated kinase; FGF: Fibroblast growth factor; TTP: Time to progression; OS: Overall survival; pERK: Phosphorylated extracellular signal-regulated kinase; PFS: Progressive-free survival; VEGFR: Vascular endothelial growth factor receptor.

Table 4 Regimens of hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

Ref.	Publishing year	Case number	Vascular invasion (%)	Regimens	Response rate (%)	Median survival time (mo)
Saeki <i>et al</i> ^[48]	2015	90	ND	Low-dose FP, including the combination of LV/IV or IV plus IFN	34.4	10.6
Nouso <i>et al</i> ^[49]	2013	476	44.1	CDDP + 5-FU	40.5	14.0 (341 patients)
Monden <i>et al</i> ^[50]	2012	34	90	IFN α , 5-FU	26.7	8.4
		35	90.3	Low-dose FP/CDDP	25.8	11.8
Yamashita <i>et al</i> ^[52]	2011	57	26.7	IFN α , CDDP, 5-FU	45.6	17.6
		57	50	IFN α , 5-FU	24.6	10.5
		102	100	IFN α , 5-FU	39.2	9
Nagano <i>et al</i> ^[57]	2011	116	100	IFN α , 5-FU	52	6.9
Obi <i>et al</i> ^[53]	2006	25	100	CDDP powder (IA call)	28	7.6
Ikeda <i>et al</i> ^[54]	2013	84	31	CDDP powder (IA call)	3.6	7.1
Iwasa <i>et al</i> ^[55]	2011	41	83.3	CDDP	12.2	7.5
Kim <i>et al</i> ^[51]	2011	97		CDDP, 5-FU	27.8	12
Yoshikawa <i>et al</i> ^[56]	2008	80	27.5	CDDP powder (IA call)	33.8	ND

ND: Not described; Low-dose FP: Low-dose 5-FU plus Cisplatin; LV: Leucovorin; IV: Isovornin; IFN: Interferon; CDDP: Cisplatin.

assessed as the difference between the baseline and 2 wk after HAIC induction (positive response: A reduction of $\geq 20\%$ from the baseline). ACTH score could stratify patients' survival (score ≤ 1 vs score ≥ 2 , 15.1 mo vs 8.7 mo; $P = 0.003$)^[48]. A validation study similarly showed that this score is useful for therapeutic assessment^[62]. Therefore, the ACTH score makes it possible to provide an early prediction of the prognosis of advanced HCC patients receiving HAIC, and can improve treatment efficiency by switching to other treatments, such as sorafenib or an experimental treatment in a clinical trial, for patients with a score ≥ 2 (Figure 1).

Modified HAIC and the combination approach

Nagamatsu *et al*^[63] developed a modified procedure for administering a low-dose FP regimen: HAIC using 5-FU after lipiodol-transcatheter arterial infusion chemotherapy (Lip-TAI) with CDDP; a multicenter phase II study showed that the MST and response rate were 27.0 mo and 75% for advanced HCC patients with portal vein thrombosis, respectively^[64]. Although this regimen produced a favorable outcome, it has not become widespread owing to the high level of proficiency needed for the procedure.

A multicenter open-labeled randomized Phase II

trial was conducted to evaluate the effect of combining the CDDP regimen of HAIC with sorafenib for treating advanced HCC. The results showed that survival was significantly better for patients receiving sorafenib plus HAIC (MST, 10.6 mo) than those receiving sorafenib alone (MST, 8.7 mo) (HR: 0.60, $P = 0.031$)^[65]; however, there was not a significant difference in survival between patients receiving sorafenib plus HAIC using low-dose FP and those receiving sorafenib alone^[66]. Therefore, further investigation is required.

Radiotherapy (RT) has become recognized as an optional treatment for HCC in the APASL and NCCN guidelines^[9,15], but it is not recommended in the AASLD and EASL guidelines^[11,13]. For advanced HCC patients with intravascular tumor thrombus, a combination of HAIC with RT is a reasonable approach. Compared to HAIC alone, a beneficial effect of 3-D conformal radiotherapy (3D-CRT) for major portal vein tumor thrombosis combined with HAIC has been demonstrated, although these results came from retrospective cohort studies^[67,68].

SORAFENIB VS HAIC

Sorafenib is recommended as a first-line treatment worldwide for advanced HCC patients (those with

Table 5 Predictive factors for response and survival of hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

Ref.	Publishing year	Case number	Regimens	Poor predictive factors for response	Poor predictive factors for survival
Saeki <i>et al</i> ^[48]	2015	90	Low-dose FP with/without LV, IV, or IV plus IFN	DCP reduction or increase of < 20% from baseline to 2 wk after HAIC	Child-Pugh B, AFP reduction or increase of < 20% from baseline to 2 wk after HAIC, DCP reduction or increase < 20% from baseline to 2 wk after HAIC
Terashima <i>et al</i> ^[58]	2015	266	IFN α , 5-FU with/without CDDP	NLR \geq 2.87 (cut-off, median value), presence of vascular invasion, presence of extrahepatic metastasis	NLR \geq 2.87 (cut-off, median value), ECOG PS 1/2, Child-Pugh score 8-9, presence of extrahepatic metastasis, CRP \geq 0.8 mg/dL, AFP \geq 235.5 ng/mL
Zaitzu <i>et al</i> ^[59]	2014	44	Low-dose FP with/without IV, or IV plus IFN	ND	Child-Pugh B, serum transferrin < 190 mg/dL
Nouso <i>et al</i> ^[49]	2013	476	CDDP + 5-FU	ND	HBs antigen positive, Child-Pugh B, tumor number > 3, tumor size > 3 cm, presence of extrahepatic metastasis, Vp3/4, AFP > 400 ng/mL
Niizeki <i>et al</i> ^[60]	2012	71	Low-dose FP	VEGF \geq 100 pg/mL	Child-Pugh B, VEGF \geq 100 pg/mL, therapeutic effect SD + PD
Miyaki <i>et al</i> ^[61]	2012	249	Low-dose FP (106 patients); IFN α , 5-FU (143 patients)	HCV antibody negative, platelet count \geq 15 \times 10 ⁴ / μ L	ECOG PS 1-2, Child-Pugh score 8-9, presence of extrahepatic metastasis, AFP \geq 1000 ng/mL, absence of additional therapy, therapeutic effect SD + PD + DO
Obi <i>et al</i> ^[53]	2006	116	IFN α , 5-FU	Not detect	Vp4, Total bilirubin \geq 1.0 mg/dL, therapeutic effect PR + SD + PD

Low-dose FP: Low-dose 5-FU plus Cisplatin; LV: Leucovorin; IV: Isovornin; IFN: Interferon; CDDP: Cisplatin; DCP: Des-gamma-carboxy prothrombin; NLR: Neutrophil-to-lymphocyte ratio; ND: Not described; VEGF: Vascular endothelial growth factor; PS: Performance status; SD: Stable disease; PD: Progressive disease; DO: Drop-out; CR: Complete response.

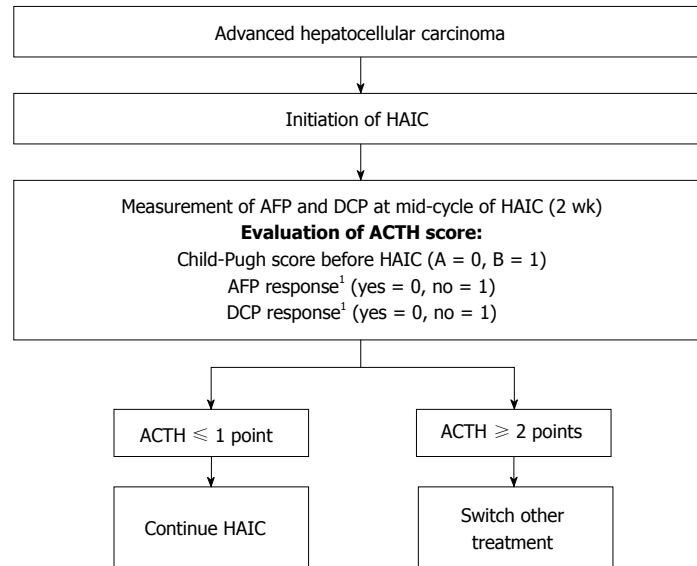


Figure 1 Treatment strategy for advanced hepatocellular carcinoma according to the hepatic arterial infusion chemotherapy score to assess continuous treatment. The score (range, 0-3) was calculated as follows: Child-Pugh score before hepatic arterial infusion chemotherapy (HAIC) (A = 0, B = 1), alpha-fetoprotein (AFP) response (yes = 0, no = 1), and des-gamma-carboxy prothrombin (DCP) response (yes = 0, no = 1). For patients with a score \leq 1, HAIC treatment would be continued, while for patients with a score \geq 2, a second-line therapy such as sorafenib and/or participation in a new clinical trial would be a better option. ¹The AFP and DCP responses were assessed 2 wk after HAIC induction; a positive response is defined as a reduction of \geq 20% from baseline. ACTH: Arterial infusion chemotherapy.

BCLC-C HCC)^[11-13]. Because of the low response rate to sorafenib, we suggest that maintaining the stability of HCC by suppressing tumor growth can significantly improve survival. Sorafenib therapy also worsens sur-

vival in patients with Child-Pugh B, unlike those with Child-Pugh A^[69]. Therefore, advanced HCC patients with Child-Pugh A are candidates for general usage of sorafenib.

Table 6 Clinical characteristics of three advanced hepatocellular carcinoma patients with complete response who have survived over 10 years

Age diagnosed as HCC	Sex	Etiology	Child-Pugh	Tumor stage ¹	Previous treatment	Maximum tumor size (mm)	Vascular invasion ¹	Regimen	Therapeutic effect	AFP (ng/mL)	DCP (mAU/mL)	HCC recurrence	Prognosis	Cause of death
67	Male	HCV	A (5)	IVA	None	110	Vp4, Vv0	Low-dose FP	CR	120700	260	62 mo	151 mo (dead)	Hepatic failure
66	Male	HCV	A (5)	III	None	50	Vp0, Vv0	Low-dose FP + IV	CR	6.4	2970	None	176 mo (dead)	Larynx cancer
44	Male	HBV	B (7)	III	None	150	Vp3, Vv3	Low-dose FP + IV + Peg IFN	CR	7145	233640	None	148 mo (alive ²)	-

¹According to the Liver Cancer Study Group of Japan; ²The follow-up period ended on January 31, 2018. HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; DCP: Des-γ-carboxyprothrombin; HCV: Hepatitis C virus; HBV: Hepatitis B virus; CR: Complete remission; Low-dose FP: Low-dose fluoropyrimidine combined with 5-FU; IV: Isovornin; Peg IFN: Pegylated interferon.

On the other hand, HAIC is not widely recommended as a standard of care for advanced HCC patients. As HAIC is thought to be one of the most effective treatment options for such patients, HAIC has become widely used in Asia, especially Japan. We propose that HAIC might be used as a treatment for achieving CR or PR. If patients with PR after HAIC receive additional therapies such as surgical resection, local ablation, or radiation, it is possible for those who show a disappearance of viable HCC to have a long survival time^[64]. In addition, although liver reserve dysfunction is a poor prognostic factor^[48,49,53,58-61], advanced HCC patients with Child-Pugh B are candidates for HAIC^[6,10].

Currently, no criteria have been established for selecting advanced HCC patients to receive either sorafenib or HAIC. According to the results of two largescale randomized controlled trials (RCTs), sorafenib indeed improved the survival of patients with macroscopic vascular invasion^[16,17]. However, these HCC patients with macroscopic vascular invasion have poorer prognoses than those without such invasion^[16,17,70,71]. Moreover, there have been no RCTs comparing sorafenib with HAIC. In a retrospective cohort study, while there was no significant difference in survival between the sorafenib group and the HAIC group, survival was significantly better in the HAIC group than in the sorafenib group among patients with macroscopic vascular invasion (14 mo vs 7 mo, $P = 0.005$)^[72]. A propensity score matched analysis also showed no significant differences in survival or disease progression between the two groups, while PFS was significantly longer in the HAIC group than in the sorafenib group, particularly for patients with portal vein invasion and/or without extrahepatic spread^[73]. On the other hand, survival was favorable in patients with HCC refractory to TACE treated with sorafenib rather than HAIC^[74]. Furthermore, it is important to preserve liver function during and after chemotherapy in advanced HCC patients. It has been reported that liver function after therapy was not significantly reduced in patients treated with HAIC compared with those treated with sorafenib^[75], and the Child-Pugh score of HAIC responders with deteriorated liver function was significantly improved after HAIC^[76]. According to our report^[62], most HAIC responders showed no deterioration of liver function. It was interesting to note that the Child-Pugh class of some responders with deteriorated live function improved from B to A after HAIC, but this did not occur in non-responders. Therefore, we conclude that HAIC may be well tolerated by advanced HCC patients with deteriorated liver function.

As of 2017, only 10 years have passed since sorafenib was first shown to be efficacious against advanced HCC. As such, it is impossible to assess survival longer than 10 years. However, we can examine survival rates from shorter-duration studies. As previously mentioned, Rimola *et al.*^[48] reported a CR rate and MST for CR patients under sorafenib of 1% and 85.8 mo, respectively. Shiba *et al.*^[77] reported that the CR rate was below 0.6% (18/3047 patients) in a nationwide study from Japan. By contrast, the CR rate for HAIC was 4.0% (19/476 patients) in a nationwide survey in Japan^[49]. According to our previous report^[78], the CR rate under HAIC using a low-dose FP-based regimen was 5% (6/114 patients), and overall 1-, 3-, 5-, 7-, and 10-year cumulative survival rates were 43.9%, 10.0%, 5.6%, 2.8%, and 2.8%, respectively (MST, 10.2 mo). Three of six CR patients from our study survived over 10 years, though 2 patients have since died and only one is still alive (Table 6 and Figure 2). Further investigations are

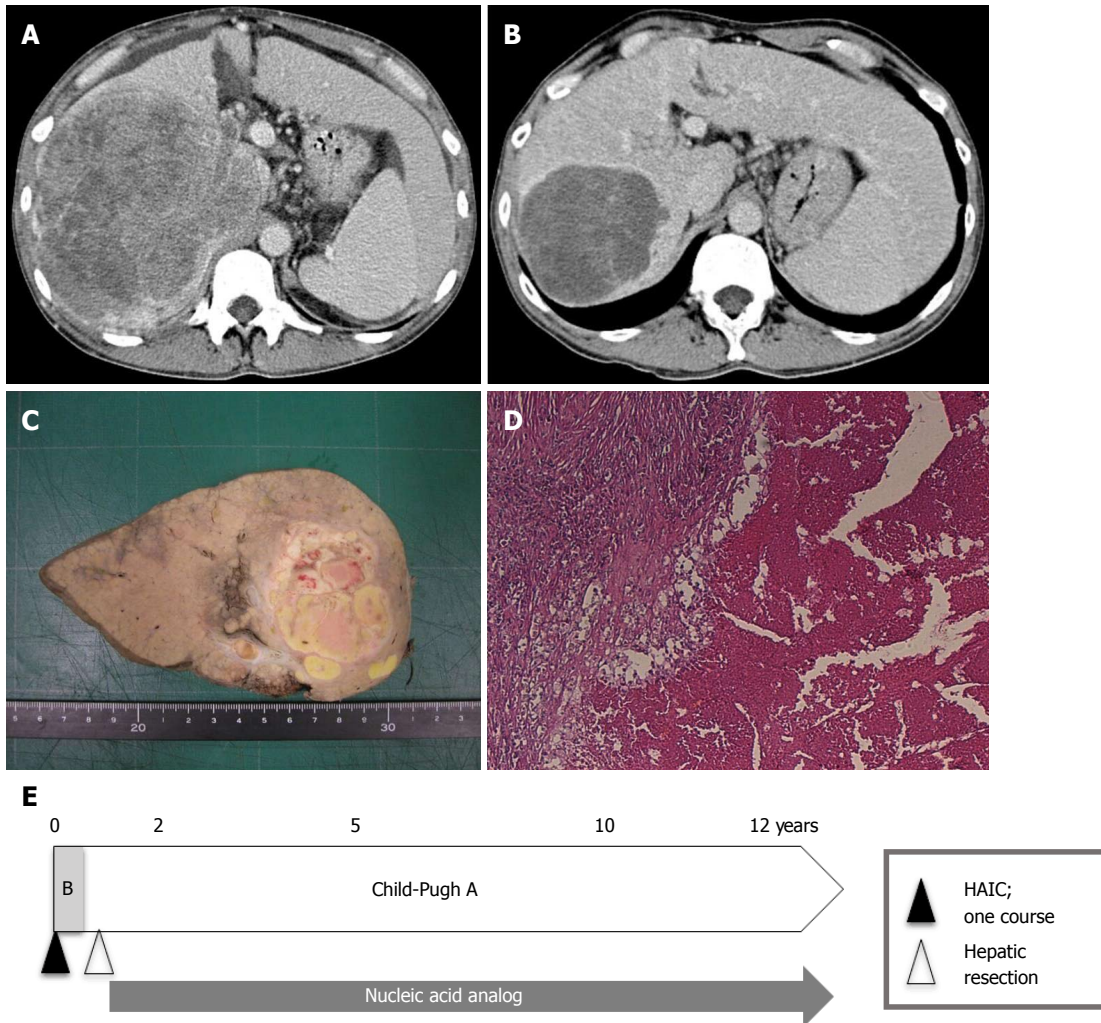


Figure 2 Patient with complete response treated with hepatic arterial infusion chemotherapy using low-dose cisplatin combined with a 5-fluorouracil (low-dose FP)-based regimen. A: This 44-year-old man had massive hepatocellular carcinoma (HCC) (16 cm in diameter) with tumor thrombosis in the right portal vein (Vp3) and the inferior vena cava (Vv3) on dynamic computed tomography; B: After one course of hepatic arterial infusion chemotherapy (HAIC), the liver tumor markedly decreased; however, as slight tumor vascularity remained, the patient was assessed as having partial response at that time; C, D: Three tumor markers [alpha-fetoprotein (AFP), des- γ -carboxyprothrombin (DCP), and AFP L3] decreased after HAIC (AFP from 7145 ng/mL to 12.7 ng/mL, DCP from 233460 mAU/mL to 51 mAU/mL, AFP L3 from 58.1% to 3.1%). The patient's Child-Pugh classification improved from B (8 points) to A (5 points). Thus, hepatic resection was performed, and histological findings showed no viable tumor cells (C, D). Finally, the patient was considered to have a complete response; E: The patient has been treated with nucleic acid analogs after the operation, and Child-Pugh A has been maintained. The patient is alive without HCC recurrence 148 mo after HAIC treatment.

required to compare long-term survival rates between sorafenib and HAIC.

Finally, we present a draft proposal of a treatment strategy for advanced HCC (Figure 3): (1) For advanced HCC patients without macroscopic vascular invasion and Child-Pugh A, the first-line treatment should be sorafenib, and second-line treatments should be either regorafenib^[21] or HAIC; (2) For advanced HCC patients with macroscopic vascular invasion and Child-Pugh A, the first-line treatment should be HAIC, and the second-line treatments should be either sorafenib or experimental treatment in clinical trials; (3) For advanced HCC patients with Child-Pugh B, the first-line treatment should be HAIC, and the second-line treatment should be clinical trials. Miyaki *et al.*^[79] reported that additional therapy with sorafenib improved the prognosis of HAIC refractory patients compared with that of patients not

treated with sorafenib therapy in a retrospective cohort study. Nonetheless, there have been no effective treatments for HAIC non-responders with deteriorated liver function (Child-Pugh B). We have shown the efficacy of an intra-arterial infusion therapy using the iron chelator deferoxamine for advanced HCC patients with deteriorated liver function^[78,80], and clinical trials are now ongoing^[81]. Because the best second-line treatment for HAIC non-responders with Child-Pugh B is to enroll in clinical trials, this remains an issue for future research.

CONCLUSION

We reviewed the current status and predictive biomarkers regarding the administration of sorafenib and HAIC for advanced HCC, and we have proposed a treatment strategy for patients with advanced HCC. The success

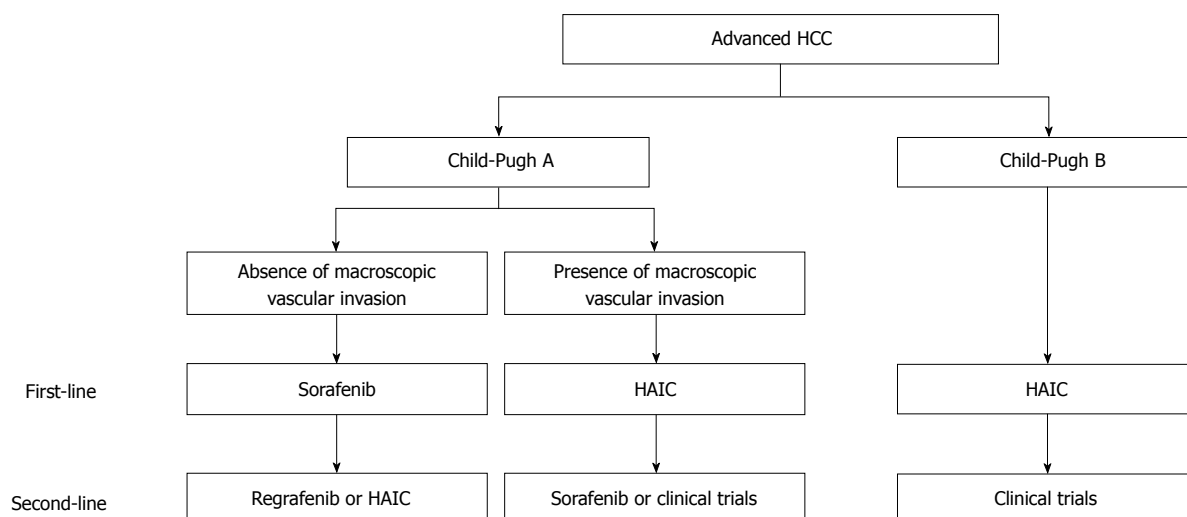


Figure 3 Draft proposal of a treatment strategy for advanced hepatocellular carcinoma. (1) For advanced hepatocellular carcinoma (HCC) patients without macroscopic vascular invasion and Child-Pugh A, the first-line treatment should be sorafenib, while second-line treatments should be either regorafenib or hepatic arterial infusion chemotherapy (HAIC); (2) For advanced HCC patients with macroscopic vascular invasion and Child-Pugh A, the first-line treatment should be HAIC, and the second-line treatments should be either sorafenib or experimental treatment in clinical trials; (3) For advanced HCC patients with Child-Pugh B, the first-line treatment should be HAIC, and the second-line treatment should be clinical trials.

of sorafenib, regorafenib, and lenvatinib in treating advanced HCC has shifted the treatment paradigm to molecular-targeted therapies. Furthermore, several immune-oncologic agents have been identified with potential for the treatment of advanced HCC^[82,83]. Thus, the chemotherapeutic interventions for advanced HCC have been kept up-to-date through several advances. However, alternative therapies will be required because of the high cost and ineffectiveness of these molecular agents for patients with deteriorated liver function.

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Current evidence on the management of hepatitis B in pregnancy

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Abstract

Hepatitis B virus (HBV) infection is one of the main public health problems across the globe, since almost one third of the world population presents serological markers of contact with the virus. A profound impact on the epidemiology has been exerted by universal vaccination programmes in many countries, nevertheless the infection is still widespread also in its active form. In the areas of high endemicity (prevalence of hepatitis B surface antigen positivity > 7%), mother-to-child transmission represents the main modality of infection spread. That makes the correct management of HBV in pregnancy a matter of utmost importance. Furthermore, the infection in pregnancy needs to be carefully assessed and handled not only with respect to the risk of vertical transmission but also with respect to gravid women health. Each therapeutic or preventive choice deserves to be weighed upon attentively. On many aspects evidence is scarce or controversial. This review will highlight the latest insights into the paramount steps in managing HBV in pregnancy, with particular attention to recommendations from recent guidelines and data from up-to-date research syntheses.

Key words: Pregnancy; Hepatitis B immunoglobulin; Hepatitis B; Therapy; Immunoprophylaxis; Antiviral prophylaxis

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Core tip: Hepatitis B is still a matter of concern worldwide. Particularly challenging is the correct management of infection during pregnancy. Two aspects have to be taken into account: The potential need to treat the mothers and, at once, the necessity to prevent

the vertical transmission of the virus to the infants. This review will discuss the most up-to-date evidence on therapeutic and preventive interventions in several scenarios characterizing the course of hepatitis B in pregnancy.

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INTRODUCTION

Despite the availability of effective preventive measures, particularly active immunization through vaccination^[1], hepatitis B virus (HBV) infection is still today a major public health issue worldwide. According to the most recent and robust estimates, 3.61% of the global population is chronically infected, as expressed by the prevalence of hepatitis B surface antigen (HBsAg)-positivity^[2]. Of course, there is a relevant heterogeneity both across and within continents and states as far as endemicity is concerned: Western Pacific Region and Africa are the world areas with the highest prevalence^[2].

Even larger is the number of people having serologic markers of previous contact with HBV: About 2 billion subjects worldwide^[3]. These individuals showing markers of resolved/occult HBV disease deserve special attention in case they undergo immunosuppressive treatment^[4]. Nevertheless, and not surprisingly, the major disease burden is related to chronic infection. In 2013, HBV was responsible for nearly 686000 deaths globally, a figure that places the virus in the top 20 causes of mortality among humans^[5]. Of note, different from other major communicable diseases, the burden of viral hepatitis, mainly driven by HBV and hepatitis C virus (HCV), has increased in terms of morbidity and mortality between 1990 and 2013^[6].

Chronic HBV infection may evolve to cirrhosis, a condition characterized by profound alteration of liver architecture and function, in about 20% of subjects and may result in hepatocellular carcinoma (HCC), as a consequence of cirrhosis itself or of viral pro-oncogenic properties^[7]. In turn, cirrhosis may be responsible for a vast array of complications: Infections^[8], mainly spontaneous bacterial peritonitis^[9] and bloodstream infections^[10], ascites^[11], hepatorenal syndrome^[12], variceal bleeding^[13].

Despite the remarkable efforts during the last decades aimed at implementing effective vaccination strategies worldwide^[14], 50 million new cases of hepatitis B are still diagnosed each year, most due to mother-to-child transmission (MTCT)^[15].

As a matter of fact, the transmission routes differ according to the entity of HBV endemicity: In areas of

high prevalence (> 7%), vertical transmission prevails, whereas in low endemic regions (prevalence < 2%), sexual transmission is the major culprit^[16]. The way and the timing of transmission are crucial factors influencing the probability of developing chronic HBV infection: Indeed, this likelihood is higher in subjects infected perinatally (up to 90%) when compared with rate of chronicity in adults after the acute phase (< 1%)^[17]. Around 15%-40% of individuals suffering from chronic hepatitis develops cirrhosis^[18].

All these figures underpin the necessity of correctly managing pregnant women with HBV infection, in order to reduce the burden of disease. Attention must be paid not only in developing countries, but also in regions such as Australia, United States, and Western Europe, where immigration from areas of high HBV endemicity may represent a challenge for physicians not accustomed to manage HBV infection in particular settings^[15]. Of course, HBV infection in pregnancy is not only a problem for infants, but also for women's health (Figure 1). In this review, the current state of the art regarding the best management of HBV in pregnancy, both for the mother and child, will be discussed.

HBV INFECTION IN PREGNANCY: FROM THE PERSPECTIVE OF GRAVID WOMEN

How HBV impacts the health of pregnant subjects

The relationship between liver diseases and pregnancy is proteiform, and three categories of pathological conditions can be described: The ones representing underlying status, pre-existing to the moment of conception; the ones coincidental with maternity; and eventually, the ones specific of pregnancy (for example, pre-eclampsia)^[19]. Viral hepatitis falls into the first two categories^[19].

As far as acute hepatitis B is concerned, its occurrence during pregnancy is not associated with higher mortality, and the related clinical picture is not distinguishable from that in the general population^[20]. This notion was further confirmed by a case-control study run in China, comparing 22 pregnant patients and 87 matched non-gravid women, all suffering from acute hepatitis B: No difference with regard to mortality and incidence of fulminant hepatitis was detected^[21]. Of note, the HBsAg loss and seroconversion rates were lower in the first group, suggesting that pregnancy might act as a risk factor for chronicity^[21].

An interesting and recent systematic review has assessed the impact of inactive HBV carriage on gravid women health, showing that this condition is not associated with complications in pregnancy, so this condition does not need any particular therapeutic measure^[22]. When it comes to chronic (active) HBV infection already established before conception, the immunological modifications that occur in pregnancy may raise the level of HBV viremia, whereas alanine

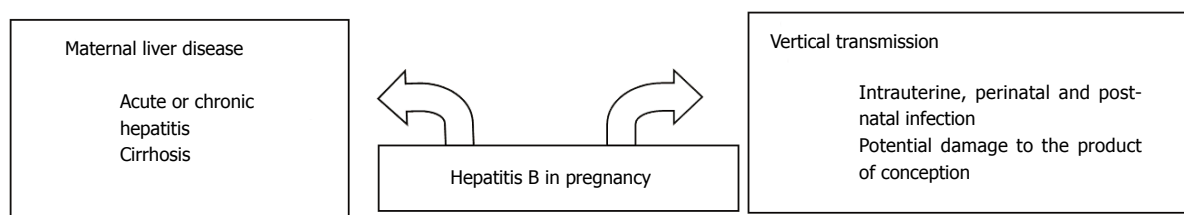


Figure 1 Hepatitis B in pregnancy: The two side of the problem.

aminotransferase (ALT) levels are normal or just above the upper limit of normal (ULN)^[23].

A more relevant exacerbation of chronic hepatitis might happen after delivery in a notable percentage of women (up to 45%), as observed in a small retrospective cohort involving 38 pregnancies in 31 subjects, in which the flare was defined as a three times increase in ALT levels within 6 mo post-partum^[24]. Authors suggested that this phenomenon, also found in the subgroup of women (8/13, 62%) who had undergone a course of lamivudine (LAM) during the third trimester, was attributable to the restoration after delivery of the immune system, whose functions were previously altered to prevent foetus rejection^[24]. The topic has been further elucidated by a subsequent prospective study recruiting 126 women: Post-partum flares, defined as ALT levels twice the ULN or the baseline, were described in 27 (25%) individuals, usually asymptomatic and with spontaneous resolution^[25]. At multivariate analysis, HBeAg positivity turned out to be the most relevant predictor of post-partum flares, although just barely not reaching the statistical significance ($P = 0.051$)^[25]. Another study, a multicentre retrospective cohort involving 101 women and 113 pregnancies, did not identify clear risk factors for exacerbation of chronic hepatitis B after delivery^[26].

With regard to maternal complications, chronic HBV infection does not seem a risk factor for many of them, as derived by research syntheses. A meta-analysis collecting data on 9088 placenta previa cases and 15571 placental abruption cases failed to demonstrate an association with HBV, implicated as a driver of an inflammation state able to induce dysfunction of trophoblasts: Odds ratio (OR) equal to 0.98 with 95%CI equal to 0.60-1.62 and OR = 1.42 with 95%CI: 0.93-2.15, respectively^[27]. A further meta-analysis involving 439514 subjects showed that HBV was not associated with increased risk of gestational diabetes mellitus (adjusted OR = 1.11, 95%CI: 0.96-1.28), a link suggested by the potential role played by the virus in inducing insulin resistance^[28]. Another research synthesis of observational studies did not detect a statistical significance between chronic HBV infection and preterm labour (OR = 1.12, 95%CI: 0.94-1.33)^[29]. More recently, a meta-analysis including 11566 women has, quite surprisingly, highlighted a negative association between chronic HBV infection and preeclampsia (OR = 0.77, 95%CI: 0.65-0.90, $P =$

0.002): Actually, the protective effect, probably due to impaired immune response and/or increased immune-tolerance caused by the virus (preeclampsia is linked with exaggerated activation of immune system), was apparent only in an Asian population, as derived from the subgroup analysis^[30].

Another aspect to be considered is how the most advanced stage of chronic liver disease, cirrhosis, impact the health of pregnant women, in the particular setting of HBV infection. Cirrhosis is, fortunately, an infrequent occurrence in pregnancy due to two factors: The development of end-stage liver disease requires time and more often takes place when women have gone beyond their reproductive age; moreover, hypothalamic-pituitary dysfunction related to cirrhosis^[31] may ensue in anovulation and amenorrhea^[32]. However, when present, cirrhosis is a relevant health issue for pregnant women. In a large population-based retrospective study in the United States on gravid women, comparing 339 cirrhotic cases with 6625 matched-controls, maternal mortality and complications of pregnancy (e.g., uterovaginal haemorrhage, pre-eclampsia, peri-partum infections) were higher among individuals suffering from liver disease: For example, the maternal death rate was 1.8% vs 0% ($P < 0.0001$)^[33]. Mortality among cirrhotic pregnant women was higher in cases of viral aetiology (HBV as well as HCV)^[34]. The high burden of liver cirrhosis in pregnancy has been confirmed in a more recent prospective study, matching 176 cirrhotic gravid women with 2179 pregnant non-cirrhotic women and 1034 cirrhotic but not pregnant female subjects^[34]. Maternal mortality rate was superior in the study group (7.8%) than in the first (0.2%) and second control group (2.5%; $P = 0.001$); variceal haemorrhage during vaginal delivery was the most frequent reason of maternal death^[34]. Indeed, the rupture of oesophageal varices represents probably the most important complications among the ones directly related to cirrhosis in pregnant women, especially in the advanced phase of pregnancy or during labour^[35]. An important predictor of liver-related complications during pregnancy is a model for end-stage liver disease (MELD) score ≥ 10 ^[36].

Treatment criteria for acute and chronic hepatitis B in pregnancy

The paramount issue is which pregnant women with HBV infection should be treated^[37].

In the case of acute hepatitis B, the main goal of the treatment should be the prevention of acute liver failure^[38]. The quality of current evidence regarding pharmacological interventions in this setting is unfortunately very low^[39]. Nevertheless, antiviral therapy is rarely necessary, since the large majority of adult patients (> 95%) have a full and spontaneous recovery^[38], and, as mentioned above, the clinical course of this entity does not differ between pregnant and non-pregnant women. The problem is the management of cases suffering from severe acute HBV infection^[40]. First, in case of serious hepatitis affecting gravid women, differential diagnosis is essential to rule out, for example, diseases unique to pregnancy, such as acute fatty liver of pregnancy (AFLP) as well as haemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, representing two hepatic emergencies in the third trimester^[40]. Once the viral aetiology is established, borrowing the recommendations applying to the general population, the treatment should rely on nucleos(t)ide analogue (NA) agents and the patients should be considered, as *extrema ratio*, for liver transplantation^[38]. Therapy with NAs can prevent acute liver failure and the related mortality^[38], but needs to be started early in the course of severe acute hepatitis, otherwise the protective effect does not display^[41]. Due to the lack of high-quality data^[39], there is high uncertainty regarding the best therapeutic options; recommendations for the general population support the use of tenofovir disoproxil fumarate (TDF), entecavir (ETV), and lamivudine (LAM)^[38]. To date, there is no information about the use in severe acute hepatitis B of tenofovir alafenamide (TAF), the prodrug of tenofovir developed to ameliorate the safety and tolerability profile of TDF^[42]. Among the above-mentioned NAs, LAM and TDF are preferable in pregnancy, in particular the latter one, owing to its high resistance barrier that is fundamental in case of prolongation of therapy for chronicity^[43]. In extreme circumstances, liver transplantation might be a therapeutic option even during pregnancy if hepatic decompensation exceeds the point of no return: The difficulty of the task is doubled, as it involves two organisms (the mother and the foetus), but successful cases have been described, also related to fulminant hepatitis B^[44].

At any rate, the management of severe acute hepatitis B in pregnancy needs more robust data to draw firm conclusions. Today, a case-by-case approach is needed. Chronic HBV infection is surely a more common scenario in pregnancy than fulminant hepatitis B. In the general population, according to the most authoritative guidelines endorsed by societies from all over the world, the main criteria for treatment are based on serum HBV DNA levels, serum ALT levels, and severity of liver disease^[38,45,46]. Despite some discrepancies (for example, the locution "inactive carriers", discouraged by Asian and European guidelines but kept in the American ones), there is a substantial consensus upon the following items, concerning situations wherein antiviral therapy is recommended: Cirrhosis; absence of cirrhosis, but

viraemia > 20000 International Units (IU)/mL and ALT levels > 2 × ULN; no cirrhosis, viraemia > 2000 IU/mL, ALT 1-2 × ULN but at least moderate to severe inflammation on liver biopsy^[38,45,46]. In the general population, there are two therapeutic options: Interferon- α (IFN α) and NAs^[16]. The rationale underpinning the choice of one strategy over another one is different. IFN α is administered to provide long-term immunological control through a finite duration treatment, attempting to achieve the so-called "functional cure" by HBsAg loss, but it is burdened by several side effects. NAs have a definitely better safety and tolerability profile, provide a very good virological control (persistent inhibition of HBV replication), but the duration of therapy is indefinite^[47].

At any rate, in pregnant women, IFN α is contraindicated; therefore the therapeutic armamentarium is limited to NAs^[48]. Among this category, currently TDF (alternatively TAF) and ETV are considered the first-line drugs when starting a new therapy for chronic HBV infection, combining an excellent safety profile with a genetic barrier of resistance higher than earlier available agents, such as LAM and telbivudine (LdT)^[38,45]. Nevertheless, according to the 5-class labelling used by the Food and Drug Administration (FDA) until 2015 as for safety in pregnancy, ETV has been classified as a "category C" agent, differently from LdT and TDF, labelled as "category B" drugs: That means the absence of teratogenic effects in animal studies^[49]. As a matter of fact, TDF is the drug of choice for pregnant females with chronic HBV infection requiring antiviral therapy (*i.e.*, advanced fibrosis and cirrhosis), also in light of the huge amount of data from the setting of gravid women under treatment against the human immunodeficiency virus (HIV), in which TDF is administered safely in combination with other antiretroviral agents throughout the pregnancy, since the first trimester^[50].

A delicate issue is how to handle cases of women who become pregnant when already on anti-HBV treatment^[51]. As a rule, appropriateness of therapy should be re-evaluated, striking a balance between benefits for the mother and the safety of the foetus^[50]. Whatever the diseases severity, IFN α must be immediately stopped; therapy with a NA can be continued in case of advanced fibrosis or cirrhosis, switching to TDF if therapy was started before conception with another drug (for instance, ETV)^[51]. In case of mild disease (*e.g.*, no advanced fibrosis, normal ALT levels, viraemia between 2000 IU/mL and 20000 IU/mL), discontinuation of therapy until delivery might be a viable option, as long as an adequate monitoring is carried out to re-start immediately treatment if necessary^[51]. Eventually, another matter of concern is the management of HBV resistance cases: In pregnant women there is limited experience, nonetheless, in gravid subjects experiencing treatment failure (HBV DNA rebound) under LAM or LdT, switching to TDF appears a safe and effective option^[52]. In Figure 2 the current knowledge regarding the treatment of HBV infection in pregnant women is summarized.




	Use of IFN in any case
	Discontinuation, until delivery, of NA agent commenced before pregnancy in case of mild hepatitis
	Use of LAM or TDF for severe acute hepatitis Use of TDF or for chronic hepatitis/cirrhosis Switching to TDF if the women was before pregnancy on treatment with other drugs

Figure 2 Treatment of acute and chronic hepatitis B in pregnancy. The column marked by the red circle refers to interventions that are not allowed. The column marked by the yellow circle refers to interventions that are backed up by low-quality or conflicting evidence. The column marked by the green circle refers to the best practice according to current evidence. IFN: Interferon; NA: Nucleos(t)ide analogue; LAM: Lamivudine; TDF: Tenofovir disoproxil fumarate.

HBV INFECTION IN PREGNANCY: FROM THE PERSPECTIVE OF FOETUSES AND NEWBORNS

Does HBV damage the product of conception?

Besides the “long-term” risks of HBV MTCT, such as chronic hepatitis, cirrhosis, and HCC^[53], physicians caring for pregnant women with HBV infection have to take into account the “short-term” potential consequences for the product of conception: For example, small for gestational age (SGA), foetal distress, preterm birth (PTB), low birth weight (LBW), congenital anomalies, and neonatal jaundice^[54].

PTB, defined as a birth occurring earlier than 37 completed weeks of gestation, represents one of the most feared complications, being worldwide the main cause of death in children under 5 years of age^[55]. A very large population-based cohort study run in China, involving 489965 women who had singleton livebirths (of whom 20827, the 4.3%, with HBV infection diagnosed before pregnancy), showed that, adjusting for several covariates, in comparison with gravid subjects without HBV infection, HBsAg positive and HBeAg negative pregnant women had a 26% higher risk of PTB, whereas in women who were both HBsAg and HBeAg positive this percentage was equal to 20%^[56]. Higher risks were observed also for early PTB (before 34 wk of gestation); unfortunately, data on viral load were not available. At any rate, the results of this recent study advocates proper medical intervention against HBV in pregnancy to improve neonatal outcomes^[56].

Another large population-based study (sample size over 2000000 people), conducted in the United States and focused on neurological complications at birth, demonstrated that women with HBV, compared with gravid subjects without HBV, had a higher likelihood to generate infants who suffered from brachial plexus injury, even after adjusting for several confounders (OR = 2.04, 95%CI: 1.15-3.60)^[57]. In a prospective cohort study in China, investigating 21004 pregnant women, of which were 513 HBV-positive and 20491

HBV-negative, no differences between the two groups were detected with regard to the rate of stillbirth, SGA and LBW, but the proportion of miscarriage was higher among gravid subjects with HBV (adjusted OR = 1.71, 95%CI: 1.23-2.38), but also in this study data on viraemia were lacking^[58].

Criteria and options for antiviral prophylaxis against HBV MTCT

The viral load is just the key factor to determine the risk of HBV MTCT^[59], that, in absence of any preventive measure, ranges from 10% to 40% when mothers are HBeAg-negative and from 70% to 90% in HBeAg-positive mothers^[60], much higher rates in comparison with the other hepatotropic virus, HCV (0%-30%)^[61].

The modalities of vertical transmission are: Intra-uterine, peripartum and post-natal infection^[59]. The transmission in utero is the most insidious route, since it represents the most important cause of passive-active immunoprophylaxis failure^[62]. There is no consensus to define correctly this occurrence (many criteria have been proposed, such as, among the many, persistent serum anti-HBc IgM positive after birth or Pre-S1 protein positivity in umbilical blood); supposed mechanisms are the passage of serum/body fluid through damaged placenta, the transmission of infected germ cells and the transfer of infected placenta or peripheral blood mononuclear cells^[62]. HBeAg-positivity is a notable driver of intrauterine transmission: The antigen can cross the placenta barrier through leakages or through infected cells, and it is linked with higher levels of HBV replication^[35]. Natal transmission during delivery represents the most impactful modality, being offspring exposed to blood or other maternal body fluids while passing the genital tract^[62]. Finally, there is postnatal infection, which encompasses all the cases in which the transmission occurs after delivery, because of contacts with maternal fluids such breast milk or blood^[62].

All guidelines, in line with the recommendations of the World Health Organization, support the administration, within 12 h of birth, of active (first dose of anti-HBV vaccine) and passive immunization through

hepatitis B immunoglobulin (HBIG) to the offspring born to HBsAg-positive mothers, a measure able to abate the rate of HBV MTC to > 90% to < 10%^[38,45,46] and supported by high-quality evidence^[63]. The paramount issue is how to avoid the failure of passive-active immunoprophylaxis, mainly ascribable to intrauterine transmission^[64]. The most recent international guidelines recommend, for gravid women not already on NAs treatment, the use of antiviral prophylaxis from week 24-28 of gestation (third trimester) if viral load > 200000 IU/mL and/or serum HBsAg levels > 4 log₁₀ IU/mL^[38,45]. The viraemia threshold was first set based on a retrospective study involving 869 mother-newborns pairs who had received proper immunoprophylaxis: Failures occurred only in infants born to HBeAg-positive women with viral load > 200000 IU/mL (maternal viraemia levels, along with detectable HBV DNA in the cord blood, was the main risk factor at multivariate analysis)^[65]. Subsequently, serum HBsAg levels emerged as a surrogate marker for viral load as well as predictive variable of HBV MTCT^[66,67].

The benefit of antiviral prophylaxis as an additional measure to HBIG and vaccination was clear in a meta-analysis of 26 studies involving 3622 pregnant women, with a risk ratio equal to 0.3; the use of LdT, LAM, and TDF turned out to be safe^[68]. There is no randomized controlled trial (RCT) directly comparing NAs as for HBV MTCT prevention: A Bayesian network meta-analysis (NMA) in 2016 demonstrated greater efficacy of LdT over LAM, but TDF was not taken into account^[69]. A more recent NMA failed to demonstrate a superiority of TDF vs LAM^[70]. At any rate, TDF is the favourite choice because of its superior barrier to resistance^[38,45]. Indeed, TDF was the drug of choice for a non-randomized trial and two RTCs vs placebo published during the last 3 years^[71-73]. The first two studies demonstrated that TDF (administered throughout the last trimester of pregnancy until 1 mo post-partum) decreased significantly the rate of HBV vertical transmission in comparison with placebo in women HBeAg-positive having high viral load^[71,72]. On the contrary, the last and more recent RCT, not considered by guidelines due to publishing timing reasons, failed to detect a significant difference between the TDF group and the placebo arm (no events out of 147 newborns vs three infection out of 147 infants, respectively, $P = 0.29$)^[73]. The study protocol contemplated the administration of TDF or placebo from 28 wk of gestation to 2 mo post-delivery in HBeAg-positive women, the large majority of them having viraemia > 20000 UI/mL, and its sample size was as large as the ones of the previous studies taken altogether^[73]. Therefore, the results of this negative trial brings into question the usefulness of NAs, specifically TDF, as additional preventive measure during the last period of pregnancy and will need to be considered and put in the right perspective by the next research syntheses and guidelines. One of the possible explanation is the very early administration of

HBV vaccination (the median time was just 1.2 h after birth)^[74].

Pending new compelling evidence, the combination of HBIG and vaccine at birth is the mainstay of HBV MTCT prevention in newborns; antiviral prophylaxis in late pregnancy may be considered for HBeAg-positive gravid women with high viral load^[75]. Furthermore, the absence of harm, weighing risks and benefits, might tip the scale in favour of NAs administration during the last trimester. The alarm raised by a case-control study (74 TDF-exposed and 69 TDF-unexposed infants) about the risk of lower neonatal bone mineral content (difference equal to 12% at 1 mo of birth) because of TDF during late pregnancy^[76] has been refuted by subsequent work (conducted by the same study group) on 509 children: At 2 years of age TDF was not linked with lower length or head circumference^[77]. This is in accordance with evidence from research synthesis confirming the safety, both for mothers and their offspring, of TDF use in pregnancy^[78]. If administered, there is no consensus about when to stop prophylaxis. Some guidelines support its prolongation until 12 wk after delivery^[38], others until 4 wk post-partum^[45]: The protocol of main trials about TDF provided for the use of drug for 4^[71,72] or 8^[73] wk after delivery. The point is to strike a balance between the potential risk of interfering with breastfeeding and the benefit on possible post-partum hepatitis flares^[35]. More conservative recommendations^[45] rely on a prospective study recruiting 91 women (101 pregnancies), showing no advantages in terms of hepatitis flare rate for gravid subjects who extended antiviral prophylaxis with TDF beyond 4 wk after delivery^[79]. Nevertheless, prolongation of antiviral prophylaxis^[38] might be useful at least for women with elevated ALT during pregnancy, since they present a higher risk of post-partum hepatitis flare, as showed by a Chinese study wherein mothers were administered LdT^[80]. With regard to other preventive strategies, unfortunately there is high uncertainty, also due to very low available evidence, upon the potential benefits of the antenatal administration of HBIG, to exploit the maternofetal diffusion through the placenta, which reaches its peak during the third trimester^[81].

Prevention of HBV MTCT: Beyond pharmacological options

The last issues involve the following topics: Delivery modalities, invasive procedures during pregnancy and breastfeeding^[38,45]. There is a huge debate about the efficacy of caesarean section (C-section) as a preventive measure. Guidelines do not back its elective implementation^[45], although meta-analyses reveal that C-section, compared with vaginal delivery, significantly decrease the risk of HBV vertical transmission^[82,83]. The problem is the high heterogeneity of the studies whose results have been retrieved and analysed by these research syntheses, one collecting data from 10 studies^[82], and the most recent from only Chinese




	Avoiding lactation
	Antiviral prophylaxis with TDF during the third trimester (until 4-12 wk after delivery) in case of maternal viral load > 200000 IU/mL C-section
	HBIG and vaccination at birth as early as possible (in newborns)

Figure 3 Prevention of hepatitis B virus vertical transmission. The column marked by the red circle refers to interventions that are not allowed. The column marked by the yellow circle refers to interventions that are backed up by low-quality or conflicting evidence. The column marked by the green circle refers to the best practice according to current evidence. TDF: Tenofovir disoproxil fumarate; IU: International units; C-section: Caesarean section; HBIG: Hepatitis B immunoglobulin.

datasets^[83]. These relevant limitations advocate well designed studies to be performed in order to shed light on this matter.

Unfortunately, there is scarce evidence regarding the best practice when invasive procedures are carried out. As far as amniocentesis is concerned, a quite recent matched case-control study (63 infants whose HBsAg-positive mothers had underwent the procedure and 198 newborns whose HBsAg-positive mothers had not underwent amniocentesis) found that HBV MTCT was more frequent among cases (6.35% vs 2.53%; $P = 0.226$); notably, the difference was apparent when maternal viral load was taken into account, especially above the threshold of 200000 IU/mL (50% vs 4.5%, $P = 0.006$)^[84]. Neither cases nor controls were born to mothers who were administered antiviral prophylaxis during pregnancy^[84]. No strong recommendations can be drawn on this basis; therefore, while waiting for studies that will investigate the potential role of antiviral prophylaxis in women with high viraemia undergoing amniocentesis, guidelines suggest that a careful assessment of harms and benefits of the invasive procedure is necessary^[45].

The last topic is breastfeeding. On one hand, lactating is allowed as long as the standard measures of passive/active prophylaxis are taken^[38,45,46]. On the other hand, there are some concerns about the safety of NAs, particularly TDF, during breastfeeding^[38,45,46]. Experiences in the HIV field indicate that antivirals are well tolerated^[85] and in particular TDF appears to be safe as to infant outcomes^[86]. In Figure 3, a summary of the current knowledge regarding the HBV MTCT prevention is depicted.

CONCLUSION

When facing HBV in pregnancy, there are two different problems to address: The first is represented by the maternal liver disease, the second by the risk of MTCT. The two issues are actually strictly inter-connected, but choices regarding potential antiviral use can profoundly differ, especially as for timing. Unfortunately, to date many questions present answers backed up

by low-quality evidence, a not rare occurrence when pregnancy is involved. For instance, it is not simple to set up large and multicentre RCTs in this setting. Moreover, there is a constant need to take carefully into account benefits and harms of each intervention, potentially impacting not only one but two lives. Regarding the first issue, in essence the indications for treatment of general population also apply to pregnant women. The drug of choice is represented by TDF; in case the gravid subjects are already on treatment with another NA, a switch is advised. IFN is absolutely contraindicated.

As to the second issue, the only mandatory measure, underpinned by incontrovertible evidence, is represented by providing passive and active immunoprophylaxis to the newborns, starting the schedule as early as possible at birth. The use of antivirals as a preventive weapon, for women not falling in the categories that require treatment, is recommended during the last trimester (until 4-12 wk after delivery) just in case of high viraemia (> 200000 UI/mL), but evidence collected so far is not solid. The drug of choice also in this case is TDF, although there is no direct or indirect proof of superiority over other NAs allowed in pregnancy; nevertheless, among them it shows the highest barrier to resistance. To date there are no data regarding the use of TAF in pregnancy, which could represent an important option, combining the same efficacy with a better safety profile.

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Hepatocellular carcinoma occurrence in DAA-treated hepatitis C virus patients: Correlated or incidental? A brief review

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Abstract

Hepatitis C virus (HCV) chronic infection induces liver fibrosis and cirrhosis but is also responsible for a significant portion of hepatocellular carcinoma (HCC) occurrence. Since it was recognized as a causative factor of chronic hepatitis, there have been multiple efforts towards viral eradication, leading to the first-generation HCV treatment that was based on interferon (IFN)- α and its analogs, mainly PEGylated interferon- α (PEG IFN α). Sustained virological response (SVR), defined as the absence of detectable RNA of HCV in blood serum for at least 24 wk after discontinuing the treatment, was accepted as a marker of viral clearance and was achieved in approximately one-half of patients treated with PEG IFN α regimens. Further research on the molecular biology of HCV gave rise to a new generation of drugs, the so-called direct antiviral agents (DAAs). DAA regimens, as implied by their name, interfere with the HCV genome or its products and have high SVR rates, over 90%, after just 12 wk of per os treatment. Although there are no questions about their efficacy or their universality, as they lack the contraindication for advanced liver disease that marks PEG IFN α , some reports of undesired oncologic outcomes after DAA treatment raised suspicions about possible interference of this treatment in HCC development. The purpose of the present review is to investigate the validity of these concerns based on recent clinical studies, summarize the mechanisms of action of DAAs and survey the updated data on HCV-induced liver carcinogenesis.

Key words: Hepatocellular carcinoma; Hepatitis C virus infection; Direct antiviral agents; Liver carcinogenesis; advanced fibrosis; Hepatitis C virus-induced cancer sequence; Sustained virological response

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Core tip: Inability to reach sustained virological response (SVR) and cirrhosis are independent prognostic factors for developing hepatocellular carcinoma (HCC) in hepatitis C virus (HCV) patients from the interferon (IFN) era. DAAs offer significantly better SVR rates. The first data regarding HCC occurrence after direct antiviral agent (DAA) treatment are similar to the data from patients who achieved SVR under IFN treatment. Some reports on early HCC occurrence or recurrence after DAA treatment are probably due to selection bias, as they were not reproduced in large comparative studies. DAAs can eradicate HCV, but they cannot terminate HCV-induced premalignant processes once triggered.

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 5.6% of all cancers^[1], making it the fifth most common cancer worldwide. There is an increasing trend in HCC incidence over the past two decades^[2], so today, HCC is considered the second leading cause of cancer-related death^[3]. One of the most well-known predisposing factors for HCC is chronic infection with hepatitis C virus (HCV), which is associated with a 15- to 20- fold increased risk for HCC development^[4]. HCV was first recognized as a distinct clinical entity in 1989 and has since become one of the most rapidly evolving fields of hepatology. Multiple studies have reported progression of chronic HCV infection to severe fibrosis and cirrhosis in 5%-20% of the patients in a period of 5-20 years^[2,3,5]. Once HCV-induced cirrhosis has been established, there is an estimated annual risk of 3%-7% of developing HCC^[6]. The advent of the first anti-HCV drugs, predominantly PEGylated interferon- α (PEG IFN α), was a major breakthrough in the management of HCV infection and consequently in HCC prevention, as there was no other specific treatment at this time. Sustained virological response (SVR) with combined regimens, consisting of ribavirin and PEG IFN, can be achieved in approximately 55%^[7] of patients, with some issues of tolerability and the limitation of being contraindicated in patients with decompensated

cirrhosis. A meticulous study on the molecular biology and pathophysiology of HCV infection led to the invention of newer direct antiviral agents (DAAs), dramatically changing the landscape of HCV infection such that viral hepatitis C should be considered a highly curable disease. Although the eradication of HCV infection with DAAs seems to be feasible in the majority of patients, there are a measurable number of patients who will progress to HCC despite viral clearance. There are even some reports^[8,9] that imply a correlation of HCC development with the use of newer oral regimes. In the present review, we attempt to clarify this puzzling topic based on recent reports from everyday clinical life, as well as reports on the pharmacological properties of the drugs used and the HCV-induced carcinogenesis sequence.

CURRENT DATA ON HCC OCCURRENCE IN HCV-TREATED PATIENTS

Chronic viral hepatitis, from either hepatitis B Virus (HBV) or HCV is the most significant predisposing factor for HCC. Forecasting models predict that without treatment, 14.4% of all HCV patients will develop HCC^[10]. If we keep in mind that globally, 150 million individuals are estimated to have HCV infection^[11], it is obvious that HCV eradication should be a major priority to decrease the incidence of HCC. SVR is currently accepted as the best tool to confirm viral eradication, and indeed, SVR has been shown to significantly reduce liver-related mortality, including the risk of HCC^[3,4,12].

The IFN experience

Interferon (IFN)- α was the first drug to gain approval for HCV infection, in the late 1980s. The immunomodifying, antiviral and antitumoral properties of IFN- α raised hopes that it would succeed both in HCV eradication and in HCC prevention, as it also inhibits liver cancer growth^[13]. The low SVR rates, low compliance and high toxicity reported with IFN led to the modification of the IFN molecule and the circulation of PEG-IFN in the 2000s. This modified form of recombinant human IFN- α has better absorption, prolonged half-life and better compliance, so it came into wider use in the treatment of HCV for about a decade, mainly in combination with the antiviral drug ribavirin.

Several studies^[2,5,14] have focused on the SVR rates with IFN-based regimens and the impact the SVR has on HCC occurrence. Achievement of viral clearance was associated with reduced incidence of HCC and diminishment of other liver-related events (0 in SVR patients vs 1.88 in non-SVR per 100 person-years of follow-up), as shown in an Italian multicenter study^[14]. In that paper, 920 patients with histologically proven cirrhosis were treated with monotherapy with conventional IFN- α . Although the SVR rate in that era was extremely low (124/920 or 13.5%), there was still

an obvious reduction in HCC occurrence in responders (5.6% vs 16%, $P < 0.01$). In the well-structured study of Van der Meer and colleagues, they compared long-term treatment outcomes among IFN monotherapy, IFN + ribavirin and PEG IFN + ribavirin in 5 hepatologic centers across Europe and Canada^[15]. Recruited patients had advanced fibrosis (Ishak score > 4) and were followed up for a mean period of 8.4 years. SVR was achieved in 36% of the patients, and these patients had significantly lower all-cause mortality (8.9% vs 26.1%, $P < 0.001$) and liver-specific mortality (0.23 vs 3.20/100 pts, $P < 0.001$). The 10-year cumulative HCC incidence rate was 5.1% (95%CI: 1.3%-8.9%) for responders (SVR) vs 21.8% (95%CI: 16.6%-27.0%) for non-SVR patients ($P < 0.001$). The relatively low SVR rates shown in that study may be attributed to the advanced fibrosis of the patients, as well as the high percentage of patients (47%) with anti-hepatitis B core antigen positivity. Ogawa *et al.*^[16] conducted a prospective national multicenter study on the effect of PEG IFN + ribavirin treatment on chronic HCV infection, focusing on the oncologic outcomes, specifically on the incidence of HCC. The 1013-patient sample included cirrhotic (150) and non-cirrhotic (863) individuals, with 47 patients (4.6%) developing HCC during a mean follow-up of 3.6 years. As SVR rate of 55% was found, and not surprisingly, non-cirrhotic treatment responders had a better prognosis regarding HCC occurrence: 5-year cumulative incidence rates of HCC were 1.7% in the SVR group and 7.6% in the non-SVR group ($P = 0.003$), but this effect was attenuated in the cirrhotic group (18.9% vs 39.4%, $P = 0.03$). The authors identified platelet count $< 150000/\text{mL}$ (HR = 4.04), failure of SVR (HR = 3.72), cirrhosis (HR = 3.22), male sex (HR = 2.98), age ≥ 60 years (HR = 2.81) and α -fetoprotein (α FP) above 10 ng/mL (HR = 2.50) as independent risk factors for HCC development. Similar results were shown in a retrospective study from Japan^[17], where reaching SVR after IFN-based treatment significantly reduced the 5-year HCC incidence (2.6% vs 8.2%, $P < 0.001$).

Hepatoma development after HCV treatment with IFN-based regimens was the main question of a meta-analysis by Morgan *et al.*^[18] in 2013. The authors included in their analysis data from 30 observational studies, comprising in total 31528 patients from 17 countries. Most of the studies selected stratified the patients according to fibrosis level, while 8 studies referred only to patients with advanced fibrosis and/or cirrhosis. Mean SVR rates were found to be quite low, as only 10853 patients (34.4%) achieved an SVR to treatment, while 1742 patients (or 5.5% of all patients) developed HCC during follow-up. When they adjusted HCC incidence according to the follow-up period and viral clearance, they found that hepatoma in HCV-treated patients developed at a rate of 0.33% per person-year (CI: 0.22% to 0.50%) in those who

achieved SVR, significantly lower than the 1.67% (CI: 1.15% to 2.42%) of non-responders.

The brave new world of direct antiviral agents

The study and analysis of the HCV genome led to the production of pharmacologic agents targeting specific regions of its nucleic RNA. The first drugs to come into commercial use were telaprevir and boceprevir, both protease inhibitors of the non-structural viral protein 3-4A (NS3-4A)^[19,20]. The addition of these agents to the "traditional" PEG IFN + ribavirin regimen increased SVR rates to approximately 70% and gained FDA approval for the treatment of HCV genotype 1 in 2011. A new target area (non-structural protein 5B -NS5B) was discovered, and sofosbuvir, an NS5B polymerase inhibitor^[21], was approved in 2013. Addition of sofosbuvir to the PEG IFN + ribavirin combination achieved SVR rates above 90%, and most important of all, the scheme was active against genotypes 1 and 4, while the combination of sofosbuvir and ribavirin achieved similar SVR rates in genotypes 2 and 3^[22]. The advent in late 2014 of NS5A inhibitors such as ledipasvir, in combination with sofosbuvir in a once-daily oral scheme, achieved SVR rates of 94%-99% in HCV genotype 1 patients in just 12 wk^[23]. Since then, more agents and combinations have come into use, and today, there are a variety of treatment options for HCV eradication, as shown in Figure 1.

Reaching SVR significantly reduces the risk of developing hepatoma, as was already known from the IFN era, so one may have expected that with the newer DAAs, such a risk would be minimized to an occasional event, but first reports from the wider clinical applications of the new treatments were worrying. Conti *et al.*^[9] published in 2016 a regional multicenter study of 344 consecutive cirrhotic patients treated with DAAs, 285 of them with no previous history of HCC. Although DAA therapy achieved sustained virological response in 91% of patients, during the 24-wk follow-up, 9 of these 285 patients (3.16%, 95%CI: 1.45-5.90) developed HCC, much higher than previous reports on IFN responders. The authors noted though that the new IFN-free oral regimens lacked the tolerability and toxicity limitations of IFN therapy, so patients with advanced liver disease who were not candidates for treatment before were receiving therapy now. Furthermore, patients were examined solely by ultrasound before the initiation of treatment, increasing the possibility of small HCC nodules escaping notice. One other study, from Spain^[8], that focused on early HCC recurrence in DAA-treated HCV patients expressed similar concerns, and a few months later, the announcement of preliminary findings by Kozbial *et al.*^[24] added to the cloud of suspicion. In a way, all these reports could be considered preliminary, keeping in mind that the new oral IFN-free regimens came into wide clinical use in 2015.

Larger studies were published last year, focusing

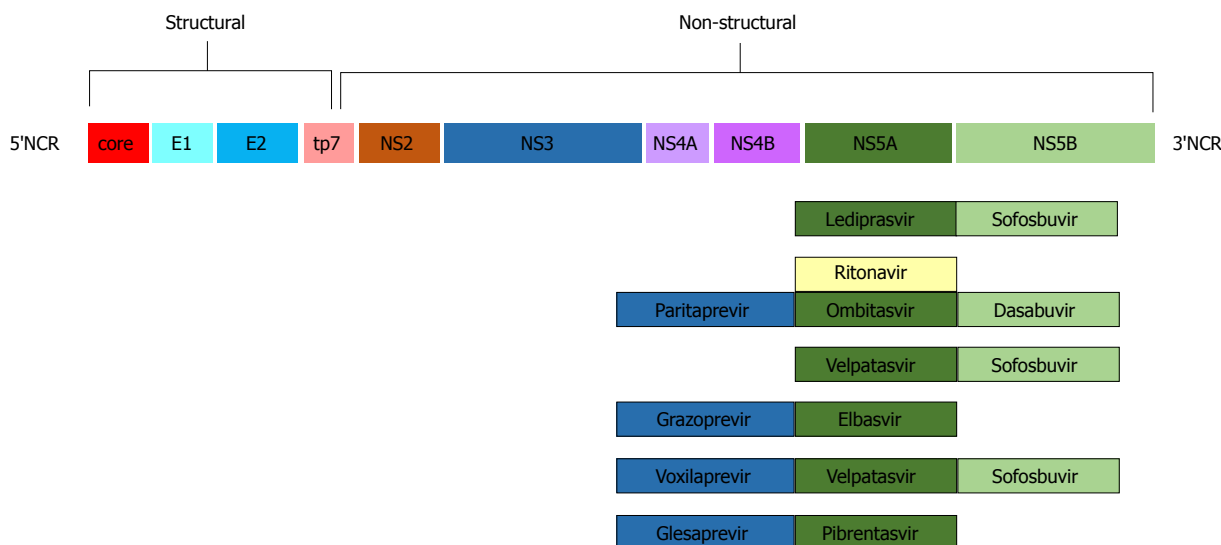


Figure 1 Current (March 2018) common direct antiviral agent regimes used in hepatitis C virus infection according to their target molecules in the hepatitis C virus genome.

on the concerns of carcinogenesis in DAA-treated patients. A big retrospective study from 129 Veteran Health Hospitals in the United States^[25] reported an annual incidence of HCC of 0.9% in patients who accomplished viral clearance after treatment with DAAs. Approximately 40% of the patients included had cirrhosis at the initiation of treatment, and approximately one-half had significant comorbidities; that may be the reason that SVR rates were relatively low (86.7%). In subgroup analysis, however, non-cirrhotic patients who achieved SVR had an even lower annual incidence of HCC, at 0.34%, while the cirrhotic responders presented an annual incidence of 1.8%, comparable to that of non-responders irrespectively of cirrhosis status (3.45%).

The same team mentioned in the IFN era led by Dr. Ogawa^[26] published a retrospective multicenter analysis in 2017 regarding early HCC development in patients who responded to DAA treatment. They reported an annual incidence of *de novo* hepatoma of 0.4% in non-cirrhotic patients, while in the cirrhotic ones HCC developed at an annual rate of 4.9%. End-of-treatment levels of α FP above 9.0 ng/mL in the individuals who were already cirrhotic before treatment, as well as low platelet count (< 150000/mL) and advanced fibrosis in the non-cirrhotic patients, were recognized as independent predictors of *de novo* HCC.

Recent comparative studies

As expected, research articles comparing the oncologic outcomes of IFN-based vs IFN-free regimens in HCV-infected persons were published in 2017. In the study of Nagata *et al.*^[17], 1897 patients treated for HCV over a 12-year period were analyzed, regardless of previous history of HCC. Although the IFN-free group of patients had worse baseline characteristics (advanced fibrosis/cirrhosis, older), the SVR rates were 65% for the IFN-

based group and 96% for the IFN-free group ($P < 0.001$). The relatively high SVR rate for the IFN-based group can probably be attributed to the fact that 1/3 of the patients received DAAs in combination with PEG IFN. The 5-year incidence rates were 2.6% (SVR) vs 8.2% (non-SVR) for the IFN group ($P < 0.001$), and the 3-year incidence rates for the DAA-only group were 3.3% vs 5.9%, respectively ($P = 0.031$). In propensity score-matched analysis, no significant differences were found in HCC occurrence ($P = 0.49$). The authors reported that post-treatment α FP and *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA⁺M2BP) could be used as independent prognostic factors of HCC development in patients achieving viral clearance.

Viral clearance by DAA treatment was associated with a 71% reduction in HCC risk in the large retrospective study by Ioannou *et al.*^[27]. The 62354 patients included were stratified in three subgroups according to HCV treatment: IFN only, IFN + DAA, and DAA only. Among patients in whom treatment succeeded (SVR), HCC developed in 2.5% of the IFN, 2.1% of the IFN+DAA group and 1.4% of the DAA group, but the follow-up period was shorter for the DAA-containing groups. Conversion to HCC incidence per patient-year showed a slight increase in hepatoma development in the DAA-only group, and this was due to the high prevalence of other risk factors that these patients had, such as cirrhosis, older age, diabetes and low serum albumin.

HCV patients with more advanced disease or other significant risk factors that were previously excluded for IFN therapy but now became candidates for treatment with DAAs probably create an unavoidable selection bias. To investigate this issue further, Waziry and colleagues^[28] performed a meta-analysis of HCC

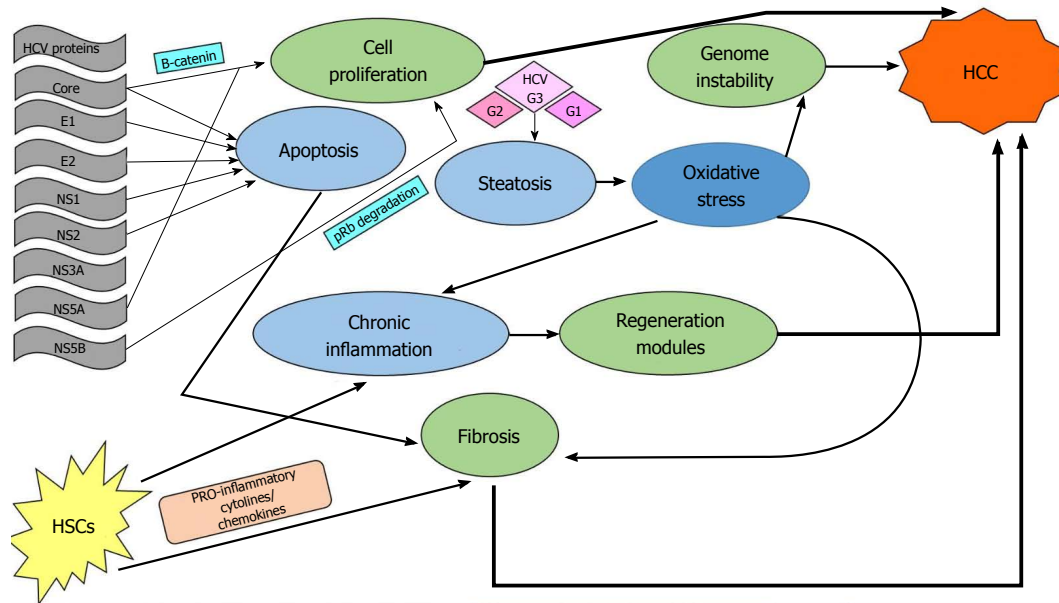


Figure 2 Schematic illustrations of identified pathways in hepatitis C virus-induced liver carcinogenesis. Viral DNA components promote host cell apoptosis deregulation, while certain viral proteins trigger proliferation signaling in the hepatic cell: Core and NS5A through the β -catenin pathway and NS5B by triggering tumor suppressor protein (pRb) degradation. Core, E1, E2, NS1 and NS2 proteins induce apoptosis, forcing a regeneration process, thereby promoting fibrosis. Oxidative stress due to inflammation also facilitates host cell genome instability and fibrosis. Inflammation activates hepatic stellate cells (HSCs) that in response secrete cytokines and chemokines, further promoting the inflammation, damage and regeneration cycle. HSCs also play a crucial role in fibrosis progression, as under chronic activation they switch their phenotype to matrix-secreting fibroblasts. Additionally, hepatitis C virus genotype 3 (as well as genotypes 1 and 2) induces steatosis, which further extends the oxidative stress, leading to earlier fibrosis. HCC: Hepatocellular carcinoma; HSCs: Hepatic stellate cells; HCV: Hepatitis C virus.

development following DAA therapy, including 17 studies with IFN treatment and 9 studies with DAA treatment. HCC occurrence in patient-years was estimated to be higher in DAA patients than in IFN-treated individuals because patients from the IFN-free group were followed for a shorter period and were older. Adjusted meta-regression analysis showed a relative risk of *de novo* HCC in DAA-treated patients vs IFN-treated of 0.67 (95%CI: 0.16-2.77, $P = 0.56$).

HCV INDUCED LIVER CARCINOGENESIS PATHWAYS

Relevant literature supports the concept that the occurrence of hepatocellular carcinoma is a multitasking process that occurs silently over years in patients with chronic HCV infection. Possible pathways include direct viral effects on the hepatic cells, immune-mediated genetic alterations, and stromal involvement via the fibrosis route. A schematic review of these pathways can be seen in Figure 2.

There is strong evidence that certain viral proteins trigger proliferating signaling in the hepatic cell, especially by the β -catenin pathway^[29]. The Core and NS5A proteins are thought to play key roles in that interference, while NS5B has been found to trigger tumor suppressor protein degradation^[30]. Additionally, viral proteins such as Core, E1, E2, NS1, and NS2 seem to induce the normal apoptotic pathway^[31].

Immune-mediated effects of HCV infection comprise a complex pathway in liver carcinogenesis. In addition to the immediate effect of infection, which is the development of chronic inflammation and consequent regeneration^[29], there is an accumulative effect on the host genome that progressively changes the hepatocellular phenotype^[32]. Stromal involvement in the inflammatory process is mediated by activation of hepatic stellate cells (HSCs), which initially secrete cytokines and chemokines that further promote the inflammation/damage/regeneration cycle^[30]. Activated HSCs become matrix-secreting myofibroblasts promoting liver fibrosis and therefore play a crucial role in cancer initiation^[30,33]. Furthermore, certain HCV genotypes, mainly genotype 3 but also genotypes 1 and 2, induce viral steatohepatitis, probably through the action of viral Core protein^[34]. Steatosis extends the oxidative stress induced by HCV infection itself^[35], and once again, HSCs are activated, triggering collagen production and fibrosis^[30].

From all the above, it becomes clear that there are distinct different pathways through which an HCV infection can induce HCC. It should be underlined, however, that some biologic cascades, once triggered, can go on independently of viral presence.

DISCUSSION

Nearly all studies regarding HCC incidence in HCV patients show 2 specific factors predicting unfavor-

able outcomes: cirrhosis and inability to reach a SVR. Which one of the two has the biggest impact on liver carcinogenesis may be suggested by the findings of Ioannou and colleagues^[27]: The occurrence of hepatocellular carcinoma, regardless of treatment, was lowest in the non-cirrhotic responders (0.24), second lowest in non-cirrhotic non-responders (0.87), second highest among cirrhotic responders (1.97) and highest, as expected, in cirrhotic non-SVR patients (3.25). Based on this, one could rather safely suggest that presence of cirrhosis affects liver carcinogenesis more robustly than the HCV virus itself.

In the present review, we decided not to focus on recurrence of HCC in HCV patients treated with IFN-free regimens, for reasons associated with the oncogenic sequence mentioned above. To be more specific, the liver of a patient who has already developed a hepatoma due to HCV is probably in a diffuse precancerous state, meaning there are quite likely some other premalignant lesions, although they are not evident. This could be the reason that occurrence and recurrence of HCC after DAA therapy seem to occur quite early, presumably in the first year. Viral clearance may inhibit further direct oncogenic interference, but the fibrosis level as well as the regenerative process probably cannot be significantly reversed. Some authors believe that the sudden decrease in viral load achieved with DAAs causes immune distortion^[36], deregulating the anti-tumor response and therefore releasing precancerous foci from immune surveillance. There are some data supporting this hypothesis, as DAA-induced SVR is associated with loss of intrahepatic immune activation by IFN^[37], yet there is no evidence of direct or indirect oncogenic effects of direct antiviral regimens. On the other hand, Abdelaziz *et al.*^[38] conducted a retrospective analysis on HCC occurrence vs recurrence in DAA-treated patients. They found that *de novo* lesions had significantly better response rates to ablation. That could be interpreted as either that the recurrent tumors are less resistant or that DAA therapy somehow interferes with the malignant potential of *de novo* lesions.

IFN has shown antitumor properties^[39], apart from its anti-inflammatory properties, that probably suppress up to a point the malignant growth in some patients. Whether there would be a benefit to prescribing IFN to patients who reached a sustained virologic response after IFN-free treatment, based on prognostic factors such as end-of-treatment α FP, low platelet count or low serum albumin, should be further investigated. Unfortunately, tolerability and contraindications limit the percentage of HCV patients who would benefit from such a tumor-preventive IFN maintenance.

CONCLUSION

Direct antiviral agents can radically change the landscape of HCV infection, as they achieve great SVR

rates with excellent patient compliance. SVR is a well-recognized risk-reducing factor for HCC development, and early studies confirm this positive effect of DAA treatment. Although there are a few publications with unexpected and undesired oncologic outcomes after IFN-free treatment, it could be that they represent solitary incidental reports. More studies with longer follow-up are needed to investigate this topic. The new drugs seem to live up to their promise, so they deserve time to show their long-term effects in real life.

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Progression and status of antiviral monitoring in patients with chronic hepatitis B: From HBsAg to HBV RNA

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Abstract

As alternative indexes of hepatitis B virus (HBV), co-

valently closed circular DNA (cccDNA) transcriptional activity, hepatitis B surface antigen (HBsAg), hepatitis B core-related antigen (HBcrAg), and peripheral blood RNA known as pgRNA, have been advocated as novel serum markers for prediction of prognosis and treatment response in chronic hepatitis B (CHB). Since the availability of commercial quantitative assays of HBsAg in 2011, HBsAg has been widely used for predicting treatment response of patients with CHB. Patients who received interferon therapy have shown a sharper reduction of HBsAg level than those who received nucleoside drug (NAs) therapy. Upon peginterferon treatment, sustained responders have presented a larger reduction of HBsAg level than the non-responders. An absence of HBsAg decline, together with < 2log reduction in HBV DNA at week 12, can serve as a stopping rule in HBsAg-negative patients infected with genotype D HBV. A sharp reduction of HBsAg titer in the NAs therapy is a predictor of HBsAg clearance in long-term treatment. HBcrAg, which consists of three species of related proteins sharing an identical 149 amino acid sequence, including HbcAg, hepatitis B e antigen (HBeAg), and a truncated 22-kDa precore protein, is still detectable in situations where serum HBV DNA levels become undetectable or HBsAg loss is achieved. Therefore, HBcrAg remains a measurable serum marker to correlate with cccDNA in this situation. The decline in HBcrAg has been observed with NAs therapy and the pattern of decline might provide prognostic information on the risk of HBV post-treatment reactivation. Peripheral blood RNA, which is known as pgRNA, directly derives from cccDNA and reflects intrahepatic cccDNA level. Quantitative pgRNA has been suggested to be helpful in CHB management. However, commercial quantitative assays are lacking. Additionally, the use of simultaneous and continuous clearance of HBV RNA and HBV DNA in serum has been suggested to be a safe stopping rule of NAs therapy for patients with CHB. However, clinical studies of large sample sizes are needed to prove the feasibility and

significance of using serum HBV RNA as the assessment standard of antiviral therapy in CHB and the safety of the stopping rule in clinics.

Key words: Hepatitis B core-related antigen; Response prediction; Chronic hepatitis B; Interferon; Nucleos(t)ide analogs; Hepatitis B surface antigen; Progenome RNA

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Core tip: As the surrogate biomarkers of intrahepatic viral replicative activity, hepatitis B surface antigen (HBsAg), hepatitis B core-related antigen (HBcrAg), and serum hepatitis B virus (HBV) RNA levels have been advocated as novel serum markers for treatment response in chronic hepatitis B. Currently, quantitative HBsAg has been widely used for predicting treatment response of chronic hepatitis B. HBcrAg can predict the risk of post-treatment reactivation of HBV as it is detectable in patients whose HBV DNA levels are undetectable or HBsAg loss is achieved. Serum HBV RNA may be useful in monitoring drug withdrawal, but clinical studies with large sample sizes remain necessary to further determine this capability.

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(9): 603-611 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v10/i9/603.htm> DOI: <http://dx.doi.org/10.4254/wjh.v10.i9.603>

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is one of the threats to human health and has become a global health issue. Approximately 0.35 billion of the worldwide population is infected with HBV, and 75% of this number is in the Asia-Pacific region. Yearly, approximately 650 000 people die of hepatic failure, liver cirrhosis, and liver cancer related to HBV infection^[1]. Antiviral drugs, which are currently approved for chronic hepatitis B (CHB) patients, include pegylated interferons (Peg-IFN- α)-2a and the following five oral polymerase inhibitors: Three nucleoside [lamivudine (LAM), telbivudine (LDT), and entecavir (ETV)] and two nucleotide [adefovir dipivoxil (ADV) and tenofovir (TDF)] drugs. Oral HBV polymerase inhibitors not only reduce the occurrence rate of corresponding complications by inhibiting HBV duplication for a long time but also increase the survival rates and living quality of patients with chronic HBV. However, these inhibitors cannot completely eliminate covalently closed circular DNA (cccDNA) molecules in hepatic cells, resulting in uncertain treatment periods. Furthermore, most patients may have to take medicines all their lives^[2,3].

Long-term virus inhibition, which is manifested by hepatitis B envelope antigen (HBeAg) seroconversion, can induce virus immunity control of some patients. Moreover, hepatitis B surface antigen (HBsAg) clearance or seroconversion may occur in some patients. Therefore, seroconversion of HBeAg and HBsAg is widely accepted as the endpoint of therapy^[2,3]. Spontaneous or therapy-induced seroconversion of HBeAg is viewed as the premise of HBsAg clearance or seroconversion, which implies the stable infection of HBV. Such seroconversion is presently acknowledged as the stopping rule^[4,5]. However, indexes for predicting seroconversion of HBeAg in antiviral therapy are not completely certain. Indexes of disease activity, namely, tissue inflammation score and alanine aminotransferase (ALT) level, and viral indexes, such as HBV DNA and HBsAg, can be used to predict seroconversion of HBeAg^[6-10]. Despite established markers, including serum HBV DNA levels and HBsAg titers, hepatitis B core-related antigen (HBcrAg) and HBV RNA are also considered serum markers of HBV infection. HBV RNA carries virus gene information, and its quantitative assay is not highly influenced by viral antigen and antibody immune compounds. Therefore, HBV RNA is important in the clinical diagnosis and response prediction. In particular, quantitative assay of serum HBV RNA level is superior to HBV DNA in terms of response prediction to HBV polymerase inhibitor based on therapy^[11-13]. Serum HBV RNA level has a key role in the prediction of the stopping rule^[14] and drug resistance^[6]. HBcrAg, which contains three related proteins that share an identical 149 amino acid sequence [HBcrAg, HBeAg, and a truncated 22-kDa precore protein (p22Cr)], is detectable in many patients with undetectable HBV DNA and HBsAg seroclearance. Along with the establishment of a fully automated detection method, HBcrAg may be extensively used in monitoring chronic HBV antiviral therapy in the future, particularly in situations where serum HBV DNA becomes undetectable. The major findings and potential clinical applications of HBcrAg in chronic HBV infection have been comprehensively described by Mak *et al.*^[15]. Therefore, this study mainly focuses on introducing the role of HBsAg titers and HBV RNA level in the antiviral therapy efficacy prediction of patients with CHB.

SIGNIFICANCE OF HBsAG LEVEL

HBsAg has been viewed as an important diagnosis index of HBV infection since its discovery by Blumberg *et al.*^[16] in 1965 and report in 1968. Recently, clinical significance of the HBsAg level has again attracted considerable attention since a corresponding quantitative assay was established and the correlation between HBsAg and cccDNA was confirmed^[17,18].

Peg-IFN- α 2a treatment

HBeAg-positive chronic hepatitis B: In HBeAg-

positive chronic hepatitis B serum, HBsAg level is closely related with intrahepatic cccDNA level and can reflect intrahepatic cccDNA contents. Reduction of HBsAg level implies a decrease in intrahepatic cccDNA^[19-21]. Responses of HBeAg-positive patients to Peg-IFN- α 2a therapy can be predicted according to HBsAg reduction. In an early study by Janssen *et al.*^[22], HBeAg-positive patients with sustained virological response (SVR) to Peg-IFN- α 2a therapy showed a dramatic reduction of serum HBsAg. In addition, the HBsAg level in patients with SVR was lower than in non-responders at the end of therapy^[22-24].

Seroconversion of HBeAg and SVR can be predicted from the baseline serum HBsAg level and its dynamic changes during Peg-IFN treatment. Early serological responses, which are defined as a low HBsAg level or a dramatic reduction of HBsAg during treatment, implies high seroconversion of HBeAg and HBV DNA suppression six months after treatment^[24-26]. Specifically, patients who presented HBsAg < 300 IU/mL and HBeAg positive at week 24 during Peg-IFN- α 2a treatment achieved an SVR of 62%, but the SVR of the remaining patients was only 11%^[25]. Patients who had HBsAg reduction > 1log₁₀ IU/mL and the absolute HBsAg < 300 IU/mL at week 24 of the therapy achieved an SVR of 75% six months after treatment. However, SVR of those patients without this combined response was only 15% ($P < 0.001$). This combined HBsAg response generated positive predictive values (PPVs) of 75% and negative predictive values (NPVs) of 85% for achieving SVR in HBeAg-positive patients.

HBsAg level decline at weeks 12 and 24 during treatment is an alternative index to predict SVR in HBeAg-positive patients and identify non-responders. In the phase III registration trial on Peg-IFN- α 2a, the PPV of HBsAg < 1500 IU/mL at week 12 and 24 on-treatment for achieving HBeAg seroconversion six months after treatment were 57% and 54% and NPV was 72% and 76%, respectively^[26]. Sonneveld *et al.*^[27] discovered that SVR of patients without any decline of HBsAg at week 12 on-treatment was only 3%. Therefore, the absence of any decline in HBsAg at week 12 generated an NPV of 97% for response prediction six months after treatment.

HBeAg-negative chronic hepatitis B: Only a few studies have discussed the baseline response predictors for peginterferon-based therapy in HBeAg-negative patients. According to the existing data, low baseline HBsAg level is associated with SVR (defined as HBV DNA < 2000 IU/mL six mo after treatment)^[28]. However, this finding has not been proven by other studies^[29,30].

Dynamic monitoring of HBsAg levels in HBeAg-negative patients treated with peginterferon may complement the predicting value of HBV DNA alone^[31-33]. Existing clinical data showed that SVR of HBeAg-negative

patients to Peg-IFN- α 2a can be predicted according to HBsAg reduction or the absolute level at week 12 or 24 during treatment. If HBsAg level decreased by 0.5log₁₀ IU/mL at week 12 and 1log₁₀ IU/mL at week 24, then the corresponding PPVs of SVR were 89% and 92% and NPVs were 90% and 97%, respectively^[30]. Retrospective analysis of dynamic HBsAg level changes in 120 HBeAg-negative patients who were enrolled into the Peg-IFN- α 2a registration study found that patients with an HBsAg level decline of more than 10% from baseline at week 12 on-treatment achieved higher virus inhibition rates than those with declines of less than 10% (47% vs 16%, $P < 0.01$)^[34]. However, HBsAg clearance occurred in a considerable proportion of patients who did not achieve more than 10% decline in HBsAg level.

Another study on Peg-IFN- α 2a therapy in HBeAg-negative patients predominantly infected with HBV genotype D indicated that dynamic monitoring of HBV DNA and HBsAg are superior to solely either marker in predicting therapeutic effects^[29]. In this study, the absence of HBsAg level decline and HBV DNA reduction of less than 2 logs after 12 wk of PEG-IFN antiviral therapy were associated with no response (defined as HBV DNA > 10 000 copies/mL and ALT remains abnormal 6 mo after treatment). This finding is accepted as a stopping rule and is verified by some studies^[29]. Nevertheless, such condition is hardly applicable to patients infected with other HBV genotypes. This result might be related with the changing influences of different genotypes on HBsAg during the treatment. Therefore, specific predictive values of different genotypes must be determined.

Additionally, a few studies have discussed the role of HBsAg level at the end of treatment in the prediction of follow-up SVR and subsequent HBsAg clearance^[35]. In this study, 52% of 23 patients with HBsAg level < 10 IU/mL at the end of treatment achieved HBsAg clearance three years after treatment, and only 2% of the remaining patients achieved HBsAg clearance. Notably, HBsAg level at the end of treatment is more important than the HBV DNA level in predicting HBsAg clearance^[34].

Conclusion: HBsAg reduction in HBeAg-positive patients at weeks 12 and 24 during Peg-IFN- α 2a therapy is conducive to the prediction of post-treatment SVR and effective identification of non-responders. Generally, HBsAg level identifies non-responders at week 12 and predicts SVR at week 24. The combined HBsAg and HBV DNA reduction in HBeAg-negative patients at week 12 can effectively recognize non-responders, especially patients infected with HBV genotype D.

NAs therapy

Variation trend of HBsAg level during NAs the-

rapy: NAs inhibits HBV replication by directly preventing HBV polymerase without affecting the synthesis of HBsAg. Selective virus gene mutation of NAs might result in changes in S open reading frame^[36]. Although no direct evidence exists for influences of these genetic changes on serum HBsAg level, these changes can certainly cause retention of intrahepatic HBsAg and increase carcinogenic risks^[37]. These factors affect the value of HBsAg quantification in predicting the antiviral efficacy of NAs. Consequently, a few studies on this topic are available, and numerous heterogeneities exist among these studies^[38-43]. Overall, serum HBsAg reduction in NAs therapy is slower and less significant compared with that in the interferon therapy^[40,41]. HBeAg-positive patients showed a larger reduction of HBsAg level than HBeAg-negative patients^[43].

HBeAg-positive chronic hepatitis B: Wursthorn *et al.*^[42] conducted a three-year follow-up observation of 162 HBeAg-positive patients on LDT treatment. After two years of treatment, all patients showed HBV DNA ≤ 60 IU/mL. Moreover, nine patients (6%) achieved HBsAg clearance. HBsAg clearance can be predicted from the sharp reduction of HBsAg ($> 1\log$) after one year of treatment. This study confirmed the importance of quantitative HBsAg monitoring in the prediction of HBsAg clearance during NAs treatment. Similar results have been obtained in the follow-up TDF studies^[44,45]. One small Chinese study disclosed that HBsAg < 100 IU/mL at the end of treatment was a sign of HBsAg seroconversion for two years after treatment^[43].

HBeAg-negative chronic hepatitis B: Among ETV- and TDF-treated patients, HBeAg-negative patients achieved a smaller reduction of HBsAg level compared with HBeAg-positive patients^[44,46]. A study in Hong Kong including 53 HBeAg-negative patients who had an average of 19 mo continuous LAM treatment and at least 12 mo post-treatment follow-up demonstrated that the end-of-treatment HBsAg titer was an independent predictor for 12 mo after treatment sustained viral suppression (HBV DNA ≤ 200 IU/mL)^[47]. All five patients with HBsAg ≤ 100 IU/mL and reduction $> 1\log$ IU/mL (PPV 100%) and four of the eight patients with either HBsAg ≤ 100 IU/mL or HBsAg reduction $> 1\log$ (PPV 50%) achieved 12 mo treatment sustained viral suppression. Moreover, other 40 patients with HBsAg reduction $\leq 1\log$ and the absolute level > 100 IU/mL were recognized as non-responders (NPV 100%) at 12 mo after treatment. HBsAg level at the end of treatment can also predict cumulative sustained response and HBsAg clearance at five years after stopping LAM.

Overall, quantitative assay of serum HBsAg in patients with CHB can provide some references regarding prediction of response to IFN and NAs thera-

pies. However, HBsAg level cannot completely reflect intrahepatic cccDNA activity, which is related with HBsAg synthesis process, quantitative assay, and antiviral drug effects. Therefore, other indexes must be combined to predict the efficacy of HBV antiviral therapy.

HBV RNA LEVEL IN PERIPHERAL BLOOD

Quantitative detection of HBV RNA in peripheral blood

The replication cycle of HBV DNA starts from end-onuclear cccDNA transcription of pre-genomic RNA (pgRNA). pgRNA is enveloped in the nucleocapsid during the formation of virus, and HBV DNA polymerase transcripts offspring DNA using pgRNA as the template. The offspring DNAs enter into the cell nucleus and facilitate virus circulation. Some offspring DNAs are assembled in the endoplasmic reticulum into complete virions and secreted from cells^[48-51]. The replicative cycle of HBV DNA shows that HBV RNA exists in cells and detecting HBV RNA in serum is difficult. In the early 1990s, some studies reported the detection of HBV RNA in peripheral blood mononuclear cells of patients infected with HBV^[51-54]. However, it was until 1996 that Köck *et al.*^[55] reported the detection of HBV RNA in peripheral blood virions of patients with chronic HBV infection using the reverse transcription PCR method. In 2001, Su *et al.*^[11] detected full-length RNA (fRNA) of HBV and truncated RNA (tRNA) in peripheral blood of patients with chronic HBV infection. They also proved that fRNA is correlated with HBeAg and HBV DNA, while tRNA is independent of HBeAg and is weakly related with HBV DNA. Subsequently, they further studied the various modes of DNA and RNA in peripheral blood in patients with CHB after a short-term LAM therapy. HBV RNA-carrying virions only account for 1% of the total virions in peripheral blood of treatment-naïve HBV-infected individuals. However, HBV RNA-carrying virions began to take the dominant role in virions after the LAM therapy. Moreover, HBV DNA level decreased more than HBV RNA level during the LAM therapy^[56,57]. Rokuhara *et al.*^[58] gained similar results from a follow-up of 24 patients with CHB on LAM treatment. They concluded that HBV RNA level in peripheral blood can reflect cccDNA level. Most recently, Huang *et al.*^[59] further examined the correlation between serum HBV RNA and intrahepatic cccDNA level. Their results indicated that serum HBV RNA reflects cccDNA activity in HBeAg-positive CHB, and total serum nucleic acids (HBV DNA plus RNA) can better reflect the activity of intrahepatic cccDNA compared with the serum HBV RNA or HBV DNA level.

Quantitative HBV RNA in peripheral blood during antiviral therapy

As an alternative index that directly reflects intrahepatic cccDNA level, HBV RNA level is one of the HBV antiviral therapy efficacy evaluation markers. HBV

RNA level can also be used to predict antiviral drug resistance and relapse after drug withdrawal^[12-14,59-61].

Peginterferon treatment

HBeAg-positive chronic hepatitis B: Peginterferon can adjust immunity and directly inhibit virus. It can also inhibit replication of virus DNA, RNA, and cccDNA. Dynamic changes of virus RNA can demonstrate the effects of Peginterferon therapy in patients with CHB. Jansen *et al.*^[12] performed dynamic monitoring on virus RNA level in peripheral blood of 13 HBeAg-positive patients on 48 wk of interferon combined with ADV therapy and two years of follow-up visits. They discovered that the baseline HBV RNA level is unrelated with response to the therapy. HBV RNA decreases less compared with HBV DNA level at different time points. HBV RNA level in combined responders (defined as HBeAg clearance, HBV DNA \leq 2000 IU/mL and normal ALT level at weeks 24 and 144 after treatment) is lower than that in non-responders at all-time points. Statistical differences exist between the two groups in terms of HBV RNA levels at all-time points after week 30 ($P < 0.001$). Therefore, responses of patients with CHB to the antiviral therapy can be predicted according to HBV RNA level. However, this study failed to disclose the threshold of prediction.

HBeAg-negative chronic hepatitis B: For HBeAg-negative patients, the HBV RNA levels of combined responders (HBV DNA \leq 2000 IU/mL and persistent ALT normalization at 24 and 144 wk of treatment-free follow-up) are lower than those of non-responders before and during the treatment ($P < 0.001$). Some combined responders showed a considerable reduction of HBV RNA in the early period. During treatment, HBV RNA level of all combined responders at week six is lower than the minimum limit of detection. On the contrary, HBV RNA level of most non-responders is higher than the minimum limit of detection ($1.8 \pm 0.2 \log_{10}$ copies/mL vs $3.7 \pm 0.7 \log_{10}$ copies/mL, $P = 0.028$) at week six. Therefore, baseline HBV RNA level is an independent predictor of responses to the therapy^[12].

Nucleos(t)ide analog treatment: HBV RNA can be detected in HBeAg-positive or -negative patients with CHB before the treatment. During the NAs therapy, the reduction rate of HBV RNA is slower than that of HBV DNA level. At the end of the follow-up (week 120), HBV RNA level in HBeAg-negative patients is lower than that in HBeAg-positive patients^[12]. HBV RNA levels of most patients are still higher than the minimum limit of detection when the HBV DNA levels are lower than the minimum limit of detection. However, HBV RNA levels in most HBeAg-negative patients are lower than the minimum limit of detection^[12].

Prediction of antiviral drug resistance and relapse

se after drug withdrawal: Given that NAs cannot directly inhibit cccDNA, many patients easily suffer from relapse after drug withdrawal. They have to take medicines for a long time, even their entire lives^[2,3]. Some patients may develop gene mutation related to drug resistance during the long-term antiviral therapy, thus resulting in the re-emergence of HBV DNA, hepatitis reflash, and even hepatic failure. Therefore, prediction of antiviral drug resistance and relapse after drug withdrawal is crucial. Existing evidence suggested that HBV RNA level can be a potential biomarker for monitoring gene mutation related to drug resistance and relapse after drug withdrawal^[14,60].

Hatakeyama *et al.*^[60] detected HBV RNA levels in peripheral blood of 7 ETV-treated patients and 36 LAM-treated patients and found that the median serum HBV RNA levels were considerably higher in patients with YMDD mutations within one year of treatment ($n = 6$, $1.788 \log$ copies/mL) than in those with YMDD mutations with more than one year of treatment ($n = 12$, $0.456 \log$ copies/mL, $P = 0.0125$) or in those without YMDD mutation ($n = 18$, $0.688 \log$ copies/mL, $P = 0.039$). The results indicated that high HBV RNA level in the early state is related with YMDD mutation.

In 2013, Tsuge *et al.*^[61] studied the correlation between HBV RNA and relapse of HBV DNA after drug withdrawal. Based on a 24-wk follow-up of 36 patients with CHB treated by NAs for at least 6 mo, 19 patients had HBV DNA relapse and 12 patients had ALT level rebound at 24 wk after discontinuation of NAs therapy. Serum total nucleic acids after three months of treatment were markedly correlated with HBV DNA rebound [odds ratio (OR) 9.474, 95% confidence interval (CI): 1.069-83.957, $P = 0.015$]. It is an independent predictor of virological recovery within 24 wk of NAs withdrawal. Wang *et al.*^[14] observed the performance of 33 patients at week 24 after NAs treatment for three or more years. All 21 HBV RNA-positive patients experienced HBV DNA rebound at the end of treatment at week 24 after drug withdrawal, but only 3 (25%) of 12 end-of-treatment HBV RNA-negative patients had virological relapse at week 24 after drug withdrawal. According to the multivariate analysis, the end-of-treatment HBV RNA level is related to virological relapse at week 24 after drug withdrawal ($P = 0.001$). Summaries of the progression and status of antiviral monitor in patients with CHB are shown in Figure 1.

CONCLUSION

In summary, HBsAg comes from either cccDNA or integrated gene fragments^[62]. HBsAg cannot completely represent the transcription activity of HBV cccDNA. HBV RNA, also known as pgRNA, only comes from cccDNA and can accurately reflect the cccDNA level. With the comprehensive knowledge on HBV RNA, the use of simultaneous continuous clearance of serum HBV DNA

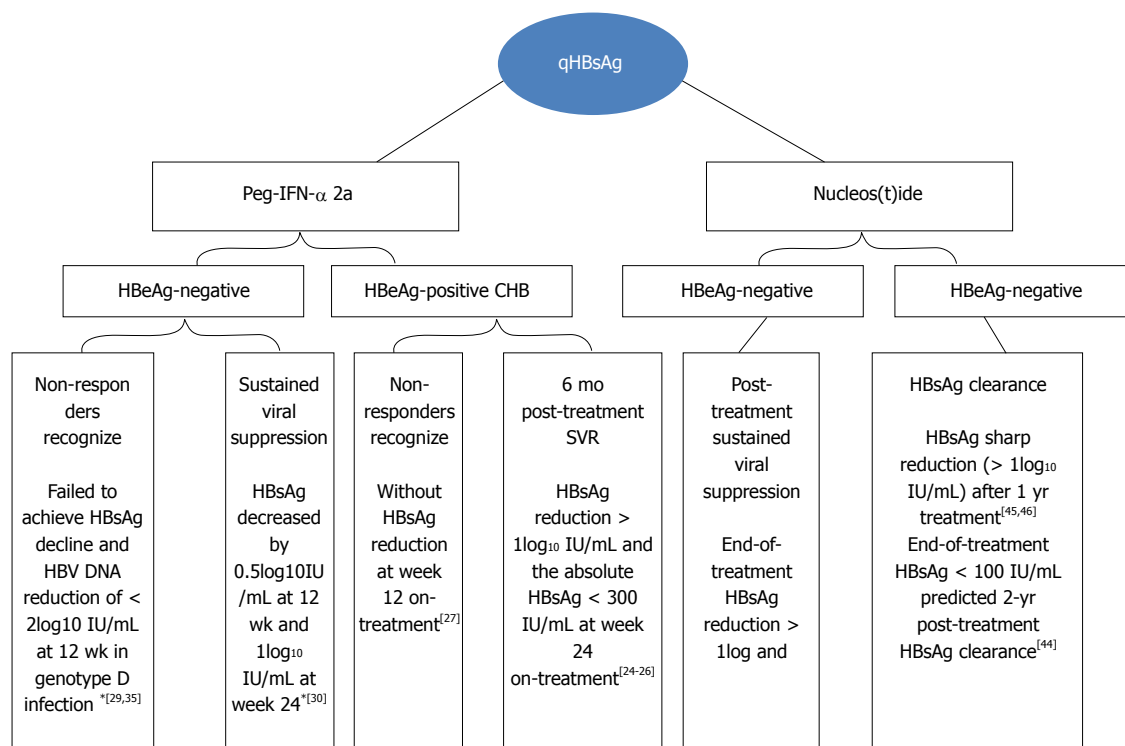
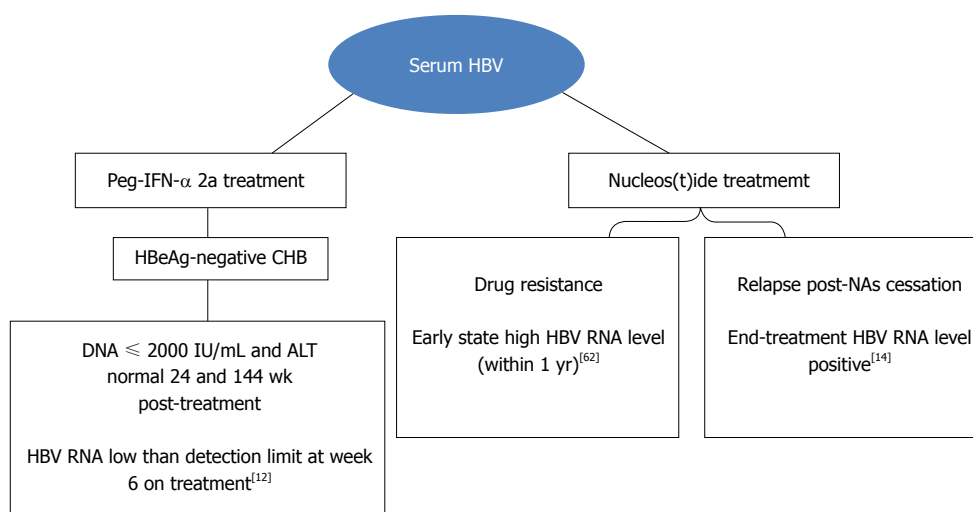
A**B**

Figure 1 Progression and status of hepatitis B surface antigen (A) and serum hepatitis B virus (B) RNA in antiviral monitor in patients with chronic hepatitis B. HBsAg: Hepatitis B surface antigen; CHB: Chronic hepatitis B; HBV: Hepatitis B virus; Peg-IFN: Pegylated interferons; ALT: Alanine aminotransferase.

and HBV RNA is suggested as the safe stopping rule in patients with CHB on NAs treatment. However, clinical evidence based on a large sample size is needed to prove the feasibility and significance of this stopping rule.

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Retrospective Study

Metabolic syndrome does not affect sustained virologic response of direct-acting antivirals while hepatitis C clearance improves hemoglobin A1c

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Abstract

AIM

To determine whether successful treatment with direc-

tacting antivirals (DAA) is associated with improvements in hemoglobin A1c (HbA1c) and if type 2 diabetes mellitus (T2DM) or metabolic syndrome affects sustained virologic response (SVR).

METHODS

We performed a retrospective analysis of all hepatitis C virus (HCV) patients at the VA Greater Los Angeles Healthcare System treated with varying DAA therapy between 2014-2016. Separate multivariable logistic regression was performed to determine predictors of HbA1c decrease ≥ 0.5 after DAA treatment and predictors of SVR 12-wk post treatment (SVR12).

RESULTS

A total of 1068 patients were treated with DAA therapy between 2014-2016. The presence of T2DM or metabolic syndrome did not adversely affect SVR12. 106 patients had both HCV and T2DM. Within that cohort, patients who achieved SVR12 had lower mean HbA1c pre treatment (7.35 *vs* 8.60, $P = 0.02$), and lower mean HbA1c post-treatment compared to non-responders (6.55 *vs* 8.61, $P = 0.01$). The mean reduction in HbA1c after treatment was greater for those who achieved SVR12 than for non-responders (0.79 *vs* 0.01, $P = 0.03$). In adjusted models, patients that achieved SVR12 were more likely to have a HbA1c decrease of ≥ 0.5 than those that did not achieve SVR12 (adjusted OR = 7.24, 95%CI: 1.22-42.94).

CONCLUSION

In HCV patients with T2DM, successful treatment with DAA was associated with a significant reduction in HbA1c suggesting that DAA may have a role in improving insulin sensitivity. Furthermore, the presence of T2DM or metabolic syndrome does not adversely affect SVR12 rates in patients treated with DAA.

Key words: Hepatitis C virus; Hemoglobin A1c; Diabetes mellitus; Direct-acting antivirals; Metabolic syndrome

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Core tip: The relationship of chronic hepatitis C virus (HCV) and type 2 diabetes mellitus is complex and lesser is known about its relationship to metabolic syndrome. While metabolic syndrome and type 2 diabetes may have had negative outcomes during the era of pegylated-interferon, research is being actively pursued to understand how direct acting antivirals (DAA) may affect these comorbidities. In this study, we show that unlike with pegylated-interferon, direct active antiviral success rates are not affected by the presence of metabolic syndrome. We further show that successful treatment of HCV with DAAs actually leads to better glycemic control 1-year post-treatment.

virologic response of direct-acting antivirals while hepatitis C clearance improves hemoglobin A1c. *World J Hepatol* 2018; 10(9): 612-621 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v10/i9/612.htm> DOI: <http://dx.doi.org/10.4254/wjh.v10.i9.612>

INTRODUCTION

Hepatitis C virus (HCV) infection is a major worldwide health problem. It is one of the most common blood-borne infections in the United States with an estimated 2.7 million people chronically infected in the United States^[1]. HCV is also one of the leading causes of cirrhosis and liver transplantation^[1,2]. There are increasing reports indicating an association between HCV and type 2 diabetes mellitus (T2DM). Individuals with HCV are more likely to have risk factors to develop T2DM and patients with T2DM have at least a 2-fold greater risk of developing HCV infection than the general population^[3,4].

Prior studies have shown that chronic HCV infection is associated with a greater risk for the development of insulin resistance^[5]. In a retrospective analysis of cirrhotic patients, those with HCV infection were 10 times more likely to have T2DM than those without HCV infection^[5]. There is evidence that patients with chronic HCV infection and increased insulin resistance have a higher prevalence of hepatic fibrosis, hepatocellular carcinoma, and other extrahepatic manifestations^[6-9]. While there are unclear mechanisms for increased insulin resistance among those with HCV, factors such as metabolic syndrome, increased hepatic iron, and serum tumor necrosis factor- α have been implicated^[10,11]. Molecular studies have also shown that the mechanism of insulin resistance can differ by HCV genotype. In particular, HCV genotype 3 has been shown to be an independent risk factor for hepatic steatosis and its viral proteins can directly interfere with intracellular insulin signaling^[12].

Previously standard therapy for HCV required the use of pegylated interferon- α (P-IFN) and ribavirin. However, these regimens had low sustained virologic response (SVR) rates and were poorly tolerated. During the era of P-IFN therapy, several studies showed that the presence of obesity and/or steatosis led to a reduction of SVR rate in HCV patients^[13,14]. In patients with diabetes and HCV who were treated with IFN-based therapies, HCV clearance was associated with improved insulin resistance and beta cell function^[15]. In 2013 and 2014, approval of newer direct acting antiviral agents (DAA) created IFN-free regimens with SVR rates greater than 90%, radically changing HCV treatment. Due to the novelty of DAA regimen, research is being actively pursued in a variety of patient populations. Within the Veterans Health Administration (VA), the incidence of chronic HCV is 2-3 times higher than the general public^[16]. Additionally, patients that receive care in the

Dong TS, Aby ES, Benhammou JN, Kawamoto J, Han SH, May FP, Pisegna JR. Metabolic syndrome does not affect sustained

VA also have a higher prevalence of obesity and T2DM compared to the general population^[17,18]. Thus, the VA presents an ideal population to evaluate the relationship between HCV and T2DM. We aim to determine if successful treatment with DAA is associated with improvements in hemoglobin A1c (HbA1c) and if the presence of T2DM or metabolic syndrome affects SVR rates.

MATERIALS AND METHODS

Study population and data collection

DAA were introduced to the VA Greater Los Angeles Healthcare System (VAGLAHS) at the beginning of April 2014. Therefore, we included all patients being treated with DAA between April 1st, 2014 and April 30th, 2016 for this study. We queried the Corporate Data Warehouse (CDW), a repository of all clinical data within the VA healthcare system, for all patients with an International Classification of Disease, Ninth Revision and/or Tenth Revision, Clinical Modification (ICD-9 CM/ICD-10 CM) diagnosis of HCV and T2DM (ICD-9: 250.00-250.93, ICD-10: E08-E13). Patients with HCV were also included if they had diabetic medications on their medication list during the study period. Patients were excluded from the cohort if they did not have SVR12 data or HbA1c one year after completion of DAA therapy. In addition to the CDW query, we manually extracted patient clinical and demographic data as well as confirm the diagnosis of T2DM from the VA electronic medical records, the Computerized Patient Record System (CPRS), of all patients screened to have both HCV and T2DM by ICD-9 CM or ICD-10 CM codes.

Outcome variables

The primary outcome was the change in HbA1c before and after DAA treatment. The secondary outcomes of interest were change in body mass index (BMI) over the same time period and if the presence of T2DM or other traits of metabolic syndrome such as hyperlipidemia (HDL), hypertension (HTN), or obesity affected SVR12. Serial BMI and HbA1c values were obtained from the year before and the year after HCV treatment and summarized as one-year pre-treatment and one-year post-treatment averages, respectively. A significant change of HbA1c was defined as a difference of 0.5 or greater, consistent with prior similar studies^[19,20]. Through chart review, we also documented whether a patient had an increase or decrease in oral hypoglycemic dose and/or injectable insulin dose from one year before to one year after treatment with DAA. A change of greater than 10% from baseline was considered a significant change in medication, similar to prior studies^[21]. Daily insulin was calculated as a total amount of basal and/or meal-time insulin over a 24-h period as documented in the patient's medication list. We also documented if there was a change in the overall number of diabetes medications from one year before to one year after treat-

ment with DAA.

Predictor variables

Data extraction included patient demographic, comorbidity, laboratory, and medication data from CPRS. Demographic data included age, sex, BMI, race, and ethnicity. For race and ethnicity, we used one mutually-exclusive variable that combined concepts of race and ethnicity by including Hispanic as a primary race (non-Hispanic white, non-Hispanic black, Hispanic, Asian, other). Comorbidities included the presence of cirrhosis by chart review, elevated Fibrosis-4 (Fib-4) score (a measure of advanced fibrosis), hyperlipidemia (HLD), hypertension (HTN), HIV, and metabolic syndrome as identified by ICD9/ICD10 codes or by chart review. A cutoff value of 3.25 was used to determine a significant Fib-4 score as consistent with prior studies^[22]. Within the cohort of patients with both HCV and T2DM, the presence of cirrhosis, ascites, and hepatic encephalopathy were confirmed by chart review of the patient's hepatology notes. If a patient had cirrhosis, clinical laboratory values were used to calculate a patient's Child-Turcotte-Pugh score and Child-Turcotte-Pugh class (CTP). Using a modified World Health Organization definition for metabolic syndrome, we defined metabolic syndrome as having at least diabetes mellitus and at least two of the following baseline characteristics as determined by ICD-9 CM codes, ICD-10 CM codes, or by medication review: A BMI ≥ 30 kg/m², HTN, and HLD^[23]. The presence of HLD was used as a surrogate for elevated triglyceride or reduced HDL cholesterol as we were unable to accurately obtain triglyceride or HDL levels for all patients before statin therapy. Furthermore, because many patients did not have urine albumin or urine creatine measurements, the presence of microalbuminuria was not analyzed. We also documented serological virologic clearance at 12-wk post treatment (SVR12), HCV genotype, and prior HCV treatment for all patients.

Statistical analysis

We performed proportions to summarize demographic and clinical characteristics and used χ^2 tests to examine differences between patients that did achieve SVR12 and those that did not. Medians were compared using the Wilcoxon rank-sum test, and means were compared using analysis of variance (ANOVA). All means are expressed with their respective standard error.

To determine predictors of SVR12, a multivariable logistic regression was performed. Due to the rare event of DAA failure, to examine the relationship between SVR12 and HbA1c, we performed univariable and multivariable penalized maximum likelihood logistic regression analyses similar to prior studies^[24,25]. Predictors included age, sex, race/ethnicity, HCV genotype, treatment experienced or naïve, DAA used, and comorbid conditions (HTN, DM, HLD, HIV, elevated Fib-4, obesity, metabolic syndrome). The reference

group was female, white, treatment experienced, low Fib-4, without HTN/HLD/metabolic syndrome, genotype 1a, and treated with sofosbuvir/simeprevir. In addition to this model, we performed sub-analyses to determine associations between SVR12 and change in insulin dose required before and after DAA therapy. Statistical assistance was provided by the Institute for Digital Research and Education at UCLA. This study along with a waiver of informed consent was approved by the VA Institutional Review Board and the Research and Development Committee at VAGLAHS.

RESULTS

Cohort characteristics

A total of 1068 patients met inclusion criteria. Baseline characteristics are summarized in Table 1. In all patients treated with DAA, SVR12 rates differ by age, Fib-4 score, HCV genotype, DAA therapy, and DAA planned duration of treatment. Patients who achieved SVR12 were older (62.0 years vs 60.7 years, $P = 0.02$). Patients with a Fib-4 score < 3.25 had an SVR12 rate of 90.9% as compared to 80.2% for patients with a Fib-4 score ≥ 3.25 . SVR12 rates were varied by HCV genotype, with genotype 2 and 3 having lower SVR12 rates than genotype 1 ($P < 0.01$). SVR12 rates also were varied by treatment and duration of therapy. SVR12 rates were highest for patients treated with shorter duration (8 and 12 wk as compared to 16 and 24 wk) and with sofosbuvir/ledipasvir (93.7%) as compared to other regimens.

Of the 1068 patients treated with DAA, 106 patients concomitantly had T2DM and HCV and at least one HbA1c value 1-year post SVR12 (Table 2). The average age was 63.2 years (0.5). Within the cohort, 105 (99.1%) were male, 29 (27.4%) were white, 39 (36.8%) were African-American, and 27 (25.5%) were Hispanic. A total of 98 patients (92.5%) achieved SVR12 and 8 patients (7.5%) did not achieve SVR12. Fifty-seven patients (53.8%) had cirrhosis with a majority (82.5%) being compensated (*i.e.*, CTP A). Seventy-two patients (67.9%) were treatment-naïve while 34 patients (32.1%) had been treated with pegylated-interferon in the past. The majority of patients had genotype 1a ($n = 60$, 56.6%) or 1b ($n = 24$, 22.6%) disease. Only 16 patients (15.1%) had a normal BMI at the time of treatment (≥ 18 and < 25) while the rest had a BMI ≥ 25 . Only 3 patients (2.8%) were co-infected with HIV. Ninety-five patients (89.6%) had HTN, 79 (74.5%) had HLD, and 85 (80.2%) had metabolic syndrome.

The average age for patients who achieved SVR12 was higher than those who did not (63.5 years vs 59.6 years, respectively, $P = 0.04$). There were no significant differences between patients who achieved SVR12 and those who did not in regards to sex, race/ethnicity, presence of cirrhosis, prior treatment experience, HCV genotype, treatment regimen, BMI, HIV status, HTN, HLD, or metabolic syndrome (Table 2).

Changes in HbA1c and BMI

Overall, average HbA1c was significantly lower after DAA therapy: 7.44% vs 6.71%, $P = 0.01$. For the subgroup of patients that achieved SVR12, the average HbA1c before treatment was significantly higher than the average after treatment (7.35% vs 6.55%, $P < 0.01$). When SVR12 was not achieved, however, HbA1c was not significantly different before and after treatment: 8.60% vs 8.61%, $P = 0.99$ (Table 3). This relationship was preserved across all genotypes with the greatest difference occurring in genotype 3 (Table 3). However, there was no difference in the change of HbA1c between genotypes or treatment regimens (Table 4).

Forty-six patients were on insulin before treatment and 43 patients were on insulin after treatment. Of those patients who were on insulin, the average daily insulin requirement before treatment was 55.1 IU (5.7) and 49.7 IU (6.2) after treatment ($P = 0.50$). For patients on insulin who achieved SVR12, the average daily insulin requirement before treatment was 55.0 IU (5.85) and the average daily insulin requirement after treatment was 48.2 IU (6.30) ($P = 0.42$). Insulin requirement also did not change significantly for patients who did not achieve SVR12 [55.5 IU (20.4) vs 58.1 IU (21.8), $P = 0.93$]. No patients analyzed were on any non-insulin injectable diabetes medications. There was no difference between the number of diabetes medications per patient before or after DAA therapy (1.23 vs 1.26, $P = 0.43$).

The study included 44 patients (41.5%) defined as overweight (BMI ≥ 25), 30 (28.3%) that were defined as obese (BMI ≥ 30), and 12 (10.4%) with severe obesity (BMI ≥ 40). The average BMI for all patients before treatment was 30.1 kg/m² (0.53), and the average BMI for all patients after treatment was 30.2 kg/m² (0.54) ($P = 0.92$). For patients who achieved SVR12, the average BMI before and after treatment were not statistically different: 30.3 kg/m² (0.56) vs 30.3 kg/m² (0.57), $P = 0.96$. Similarly, the average BMI was not different before and after treatment for the patients that did not achieve SVR12: 28.8 kg/m² (1.4) vs 29.2 kg/m² (1.5), $P = 0.92$.

SVR12 is not affected by the components of metabolic syndrome

After adjusting for age, sex, race/ethnicity, cirrhosis, treatment experience, HCV genotype, treatment regimen, HIV status, and treatment duration, the individual components of metabolic syndrome (obesity, HTN, HLD, and T2DM) and the presence of metabolic syndrome itself did not predict SVR12 (Table 5). For the full logistic regression for SVR12, please see supplemental Table 1.

SVR12 as a predictor of improved insulin resistance

When adjusting for age, sex, race/ethnicity, cirrhosis,

Table 1 Baseline characteristics of all patients treated with direct-acting antivirals *n* (%)

	All patients (<i>n</i> = 1068)	SVR12 not achieved (<i>n</i> = 138)	SVR12 achieved (<i>n</i> = 930)	<i>P</i> -value
Age (mean ± SE, yr)	61.8 ± 0.2	60.7 ± 0.5	62.0 ± 0.2	0.02
Sex				
Male	97.5 (1041)	12.8 (133)	87.2 (908)	0.78
Female	2.5 (27)	18.500 (5)	81.50 (22)	
Race/ethnicity				
White	37.5 (400)	11.00 (44)	89.0 (356)	0.14
African American	37.5 (401)	14.70 (59)	85.3 (342)	
Hispanic	15.1 (161)	16.80 (27)	83.2 (134)	
Asian	0.70 (7)	14.30 (1)	85.700 (6)	
Other	9.3 (99)	7.10 (7)	92.90 (92)	
Fib4 < 3.25	64.6 (690)	9.1 (63)	90.9 (627)	< 0.01
Fib4 ≥ 3.25	35.4 (378)	19.80 (75)	80.2 (303)	
Treatment naïve	79.5 (849)	16.4 (102)	83.6 (747)	0.09
Treatment experienced	20.5 (219)	16.40 (36)	83.6 (183)	
HCV genotype				
HCV genotype 1a	58.0 (619)	12.10 (75)	87.9 (544)	< 0.01
HCV genotype 1b	25.9 (277)	10.10 (28)	89.9 (249)	
HCV genotype 2	7.9 (84)	21.40 (18)	78.60 (66)	
HCV genotype 3	6.9 (74)	23.00 (17)	77.00 (57)	
HCV genotype 4	1.2 (13)	0.00 (0)	100.0 (13)	
HCV genotype 6	0.10 (1)	0.00 (0)	100.0 (1)	
Treatment				
PrOD	4.5 (48)	8.30 (4)	91.70 (44)	0.01
PrOD/RBV	17.6 (188)	14.40 (27)	85.6 (161)	
Sofosbuvir/Ledipasvir	34.4 (367)	6.3 (23)	93.7 (344)	
Sofosbuvir/Ledipasvir/RBV	13.4 (143)	13.30 (19)	86.7 (124)	
Sofosbuvir/RBV	9.8 (105)	23.80 (25)	76.20 (80)	
Sofosbuvir/Simeprevir	17.5 (187)	19.80 (37)	80.2 (150)	
Other regimens	2.9 (30)	10.0 (3)	90.00 (27)	
No HIV	96.7 (1033)	13.1 (135)	86.9 (898)	0.44
HIV	3.3 (35)	8.60 (3)	91.40 (32)	
Duration of treatment				
8 wk	19.9 (213)	6.6 (14)	93.4 (199)	< 0.01
12 wk	73.1 (781)	13.4 (105)	86.6 (676)	
16 wk	2.8 (30)	40.00 (12)	60.00 (18)	
24 wk	4.1 (44)	15.90 (7)	84.10 (37)	
BMI < 30	66.5 (710)	12.50 (89)	87.5 (621)	0.60
BMI > 30	33.5 (358)	13.70 (49)	86.3 (309)	
No HTN	43.7 (467)	13.30 (62)	86.7 (405)	0.76
HTN	56.3 (601)	12.60 (76)	87.5 (525)	
No T2DM	85.6 (914)	12.4 (113)	87.6 (801)	0.15
T2DM	14.4 (154)	16.20 (25)	83.8 (129)	
No HLD	58.1 (620)	14.50 (90)	85.5 (530)	0.70
HLD	41.9 (448)	10.70 (48)	89.3 (400)	
No metabolic syndrome	87.1 (930)	13.0 (121)	87.0 (809)	0.84
Metabolic syndrome	12.9 (138)	12.30 (17)	87.7 (121)	

PrODL: Ombitasvir/Paritaprevir/Ritonavir/Dasabuvir; Fib4: Fibrosis 4 score; RBV: Ribavirin; BMI: Body mass index; HTN: Hypertension; T2DM: Type 2 diabetes mellitus; HLD: Hyperlipidemia; SVR: Sustained virologic response; HCV: Hepatitis C virus.

treatment experience, HCV genotype, treatment regimen, obesity, HIV status, HTN, HLD, and metabolic syndrome, SVR12 significantly predicted a 0.5-unit improvement in HbA1c with treatment in patients with both T2DM and HCV: Adjusted OR (aOR) = 7.24 (95%CI: 1.22-42.94) (Table 6). Patients who achieved SVR12 were also significantly less likely to require an increase in insulin after HCV therapy than those who did not achieve SVR12: aOR = 0.166 (95%CI: 0.03-0.74). A decrease of HbA1c was not associated with an increase in oral hypoglycemic dose (aOR = 1.07, 95%CI: 0.48-1.35) or injectable insulin dose (aOR = 0.6, 95%CI: 0.57-1.87) after DAA therapy.

DISCUSSION

This study demonstrates that HCV clearance with DAAs is associated with a clinically significant decrease in HbA1c independent of other demographic and clinical factors such as metabolic syndrome and BMI. This data corroborates recent studies that showed similar outcomes with DAA therapy^[26]. While Hum *et al.*^[26] attempted to control for DM medication use by looking at the percent of patients taking any DM medication or by the number of DM medication class, being unable to look at dosage change or changes in medication for an individual patient was a limitation of their study.

Table 2 Baseline characteristics of patients with hepatitis C virus and type 2 diabetes mellitus

	All patients (<i>n</i> = 106)	SVR12 not achieved (<i>n</i> = 8)	SVR12 achieved (<i>n</i> = 98)	<i>P</i> -value
Age (mean ± SD, yr)	63.2 ± 0.5	59.6 ± 1.4	63.5 ± 0.5	0.04
Sex				
Male	99.1 (105)	7.60 (8)	92.40 (97)	0.74
Female	0.90 (1)	0.00 (0)	100.0 (1)	
Race/ethnicity				
White	27.40 (29)	6.90 (2)	93.20 (27)	0.15
African American	36.80 (39)	5.10 (2)	94.90 (37)	
Hispanic	25.50 (27)	11.1 (3)	88.90 (24)	
Asian	1.90 (2)	50.0 (1)	50.00 (1)	
Other	8.50 (9)	0.00 (0)	100.0 (9)	
Cirrhosis				
Yes	53.80 (57)	8.80 (5)	91.20 (52)	0.61
No	46.20 (49)	6.10 (3)	93.90 (46)	
CTP class				
CTP A	82.50 (47)	6.40 (3)	93.60 (44)	0.21
CTP B	14.00 (8)	25.0 (2)	75.0 (6)	
CTP C	3.50 (2)	0.00 (0)	100.0 (2)	
Treatment status				
Naive	67.90 (72)	5.60 (4)	94.40 (68)	0.26
Experienced	32.10 (34)	11.8 (4)	88.20 (30)	
HCV genotype				
Genotype 1a	56.60 (60)	10.0 (6)	90.00 (54)	0.67
Genotype 1b	22.60 (24)	4.20 (1)	95.80 (23)	
Genotype 2	12.30 (13)	0.00 (0)	100.0 (13)	
Genotype 3	7.60 (8)	12.5 (1)	87.50 (7)	
Genotype 4	0.90 (1)	0.00 (0)	100.0 (1)	
Treatment regimen				
Sofosbuvir/Simeprevir	39.60 (42)	7.10 (3)	92.90 (39)	0.96
Sofosbuvir/Ribavirin	17.00 (18)	5.60 (1)	94.40 (17)	
PrOD	18.90 (20)	10.0 (2)	90.00 (18)	
Sofosbuvir/Ledipasvir	24.50 (26)	7.70 (2)	92.30 (24)	
Body mass index				
Normal	15.10 (16)	6.20 (1)	93.80 (15)	0.78
Overweight	41.50 (44)	9.10 (4)	90.90 (40)	
Obese	28.30 (30)	6.70 (2)	93.30 (28)	
Severely obese	10.40 (12)	8.30 (1)	91.70 (11)	
Very severely obese	4.70 (4)	0.00 (0)	100.0 (4)	
Co-HIV infection	2.80 (3)	100.0 (3)	0.0 (0)	0.62
HTN	89.60 (95)	8.40 (8)	91.60 (87)	0.32
HLD	74.50 (79)	6.30 (5)	93.70 (74)	0.42
Metabolic syndrome	80.20 (85)	7.10 (6)	92.90 (79)	0.71

HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HTN: Hypertension; HLD: Hyperlipidemia; PrOD: Ombitasvir/Paritaprevir/Ritonavir/Dasabuvir; SVR: Sustained virologic response; CTP: Child-Turcotte-Pugh.

By analyzing each individual patient and examining both changes in the number of DM medication and the dosage, we find that the change of HbA1c was not due to an increase in oral hypoglycemic or insulin treatment. The findings imply that the change in HbA1c was most likely due to a change in host insulin resistance due to HCV clearance. This is in line with a recent study showing that HCV clearance with DAA reverses insulin resistance^[27]. Our finding, however, is contrary to what Chaudhury *et al.*^[28] described in their recent paper published in December of 2017. While their study showed no change in HbA1c with DAA therapy, only 17% of patients had diabetes and their average baseline HbA1c was 5.75%. A reduction in HbA1c in a non-diabetic patient is hard to achieve. Therefore, it is reasonable to suggest that if they only examined patients with T2DM with a baseline HbA1c

≥ 6.5% they would have found similar conclusions. Our data is further corroborated by data showing that successful treatment with DAA is also associated with a decreased likelihood of requiring higher insulin doses for DM management. This is contrary to a paper by Stine *et al.*^[29] showing that a third of patients required an increase in their DM medication after DAA therapy. However, they only examined patients up to the time of SVR12. It is possible that if they examined data one year after SVR12 they would have had similar results. Our study also shows that changes in HbA1c did not vary between HCV genotype and DAA treatment regimen, implying that HCV clearance itself plays an important role in insulin resistance. Even though the baseline HbA1c was different in those who did achieve SVR12 compared to those that did not, our study shows that metabolic syndrome or its individual components

Table 3 Mean hemoglobin A1c before and after hepatitis C virus treatment by hepatitis C virus genotype and sustained virologic response 12-wk status

Genotype	Before DAA	SE	After DAA	SE	P-value
Mean HbA1c of patients who did achieve SVR12					
1a	7.5	0.19	6.68	0.14	0.001
1b	7.3	0.22	6.59	0.21	0.03
2	7.09	0.35	6.29	0.21	0.06
3	7.12	0.37	6.10	0.39	0.08
4	5.5	NA	5.30	NA	NA
Overall	7.35	0.13	6.55	0.11	< 0.01
Mean HbA1c of patients who did not achieve SVR12					
1a	8.98	1.14	8.95	1.41	0.98
1b	6.4	NA	6.50	NA	NA
2	No observations				
3	8.5	NA	8.70	NA	NA
4	No observations				
Overall	8.6	0.89	8.61	1.08	0.99

HbA1c: Hemoglobin A1c; SVR12: Sustained virologic response 12 wk after treatment; DAA: Direct acting antiviral; SE: Standard error.

Table 4 Mean difference in hemoglobin A1c by hepatitis C virus genotype and direct acting antiviral treatment

	Average change HbA1c (SE)	P-value
HCV genotype		
1a	-0.73 (0.13)	0.97
1b	-0.69 (0.20)	
2	-0.79 (0.27)	
3	-0.88 (0.51)	
4	-0.20 (NA)	
Treatment		
Sofosbuvir/Simeprevir	-0.71 (0.16)	0.97
Sofosbuvir/Ribavirin	-0.77 (0.27)	
PrOD	-0.66 (0.15)	
Ledipasvir/Sofosbuvir	-0.80 (0.24)	

HbA1c: Hemoglobin A1c; HCV: Hepatitis C virus; PrOD: Ombitasvir/Paritaprevir/Ritonavir/ Dasabuvir

Table 5 Components of metabolic syndrome on Sustained virologic response 12 wk after treatment

	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Obese	0.90 (0.62-1.31)	1.25 (0.82-1.90)
HTN	1.06 (0.74-1.52)	0.81 (0.51-1.28)
HLD	1.42 (0.97-2.10)	1.31 (0.87-1.97)
T2DM	0.72 (0.48-1.10)	0.82 (0.55-1.09)
Metabolic syndrome	1.04 (0.68-1.60)	1.81 (0.75-4.37)

HTN: Hypertension; HLD: Hyperlipidemia; T2DM: Type 2 diabetes mellitus.

(HTN, T2DM, Obesity, HLD) does not negatively affect SVR12 rates.

Prior studies have demonstrated an association between HCV infection and host metabolism. It has been shown that the hepatitis C virus depends on host lipids for entry and replication in hepatocytes^[30-32]. HCV infection, in return, leads to a disruption of host

metabolism, which can result in insulin resistance, lipid dysregulation, and hepatic steatosis^[33].

The data represented here are consistent with data during the pegylated-interferon era where SVR clearance was associated with decreased insulin resistance and improved beta-cell function^[10,15,34,35]. Kawaguchi *et al.*^[15], for example, demonstrated a three-fold increase in insulin receptor substrate 1 and 2 expressed in hepatocytes in 29 biopsy-proven HCV infection who maintained SVR after pegylated-interferon therapy. They also demonstrated a correlation between serum ferritin and homeostatic model assessment (HOMA) of β -cell function. Their data supports prior data that shows hepatic iron-induced oxidative stress may be related to insulin resistance^[36]. Knobler *et al.*^[11] proposed an alternative mechanism in which HCV infection may mediate diabetes. In a comparison of 23 patients with DM and HCV to 28 patients with HCV alone, they showed that serum TNF- α levels were more prominent in those with concurrent DM. This is similar to prior data that shows that TNF- α signaling may be related to obesity and insulin resistance, thus suggesting a role of inflammation in creating a "diabetogenic" state^[37].

During the era of pegylated-interferon, Bressler *et al.*^[14] showed that obesity was an independent risk factor for reduced SVR12. Because pegylated-interferon is primarily taken up in the lymphatic system and because obese patients have poor lymphatic circulation, they proposed that the difference they described was due to dissimilar pharmacokinetic properties of obese and non-obese patients. While that may have been true for pegylated-interferon, our study shows that obesity, HTN, T2DM, or HLD does not significantly affect SVR12 rates of patients on DAA therapy. Unsurprisingly, SVR12 rates were reduced in patients with an elevated Fib-4 score, in patients with genotype 3, and in patients treated with suboptimal regimens such as sofosbuvir/ribavirin or sofosbuvir/simeprevir. This is similar to prior studies showing reduced SVR12 in similar treated groups^[38-40]. We further hypothesized that patients who were more ill were more likely to be treated for a longer duration thus explaining the discrepancy between SVR rates and treatment duration.

While this study demonstrates the interplay between host metabolisms with HCV infection, there are certain limitations. The study implies that HCV clearance is associated with a reduction in insulin resistance, however, we were unable to measure insulin resistance directly before and after treatment. With primary care providers preferentially using HbA1c over fasting blood glucose, we were unable to use HOMA-IR (insulin resistance), HOMA- β (β -cell function), or other indices of insulin resistance. Nonetheless, HbA1c is a well-accepted and commonly utilized method to evaluate for insulin sensitivity, and we feel that using HbA1c as an outcome is consistent with clinical diagnosis, management, and prior studies^[18,20]. Second,

Table 6 Odds ratio for a decrease of hemoglobin A1c > 0.5 among type 2 diabetes mellitus patients treated with direct acting antiviral

Predictor	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Age	1.03 (0.95-1.11)	1.07 (0.96-1.20)
Race/ethnicity		
White ¹	1.58 (0.64-3.91)	
African American	0.89 (0.32-2.43)	0.52 (0.13-2.06)
Hispanic	0.53 (0.18-1.57)	0.65 (0.16-2.66)
Asian	0.50 (0.02-8.85)	0.8 (0.04-10.01)
Other	0.83 (0.16-4.21)	0.98 (0.12-7.90)
Cirrhosis (by chart review)	0.62 (0.28-1.37)	0.59 (0.20-1.69)
Treatment naïve	0.84 (0.87-4.59)	2.61 (0.92-7.29)
HCV genotype		
1a ¹	0.77 (0.35-1.71)	
1b	1.33 (0.49-3.60)	0.79 (0.22-2.86)
2	1.50 (0.41-5.42)	6.75 (0.26-176.51)
3	0.66 (0.15-2.92)	3.81 (0.29-50.00)
Treatment regimen		
Sofosbuvir/Simeprevir ¹	1.23 (0.55-2.75)	
Sofosbuvir/Ribavirin	0.75 (0.27-2.09)	0.07 (0.003-1.50)
PrOD	1.60 (0.56-4.56)	0.71 (0.17-2.86)
Ledipasvir/Sofosbuvir	0.66 (0.27-1.62)	0.35 (0.09-1.36)
Overweight	1.36 (0.42-4.35)	0.89 (0.20-1.69)
Obese	1.55 (0.44-5.40)	1.10 (0.20-5.93)
Severely obese	1.08 (0.24-4.94)	0.41 (0.05-3.07)
Very severely obese	0.26 (0.21-3.06)	0.08 (0.003-2.07)
HTN	1.36 (0.38-4.80)	1.15 (0.15-8.86)
HLD	1.69 (0.69-4.09)	1.45 (0.29-7.26)
Metabolic syndrome	1.58 (0.60-4.15)	0.96 (0.10-9.19)
SVR12 ^a	10.67 (1.76-64.7)	7.24 (1.22-42.94)

¹Reference group; ^a*P* < 0.05. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HTN: Hypertension; HLD: Hyperlipidemia; SVR12: Sustained virologic response 12 wk post-treatment; PrOD: Ombitasvir/Paritaprevir/Ritonavir/ Dasabuvir.

our study was a single center study conducted in the VA. VA patients are predominantly male and have different socioeconomic factors that may affect health as compared to the general population. Nonetheless, GLAVA is one of the largest VA medical center that includes an integrated network of 12 sites serving a racially and ethnically diverse population in Southern California.

In conclusion, this study of a diverse VA patient population demonstrates that HCV clearance with DAAs is associated with a clinically significant decrease in HbA1c. This change was consistent across all genotypes and treatment regimens. The change in HbA1c was independent of changes in BMI and DM medication requirements and so may represent a decrease in host insulin resistance or increased insulin sensitivity. This study substantiates similar studies during the pegylated-interferon era by showing that HCV clearance irrespective of treatment regimen and genotype leads to improved DM outcomes. However, unlike prior studies during the period of pegylated-interferon, we show that HTN, HLD, T2DM, obesity, and metabolic syndrome do not negatively affect SVR12 rates for DAA. Future prospective studies analyzing patient fasting blood glucose and serum insulin should be performed to validate these findings and to help elucidate the relationship between HCV, insulin sensitivity, and longer-term DM outcomes such as cardiovascular events.

ARTICLE HIGHLIGHTS

Research background

Hepatitis C virus (HCV) infection is a major worldwide health problem. There are increasing reports indicating an association between HCV and type 2 diabetes mellitus (T2DM). Individuals with HCV are more likely to have risk factors to develop T2DM and patients with T2DM have at least a 2-fold greater risk of developing HCV infection than the general population. Previously standard therapy for HCV required the use of pegylated interferon- α (P-IFN) and ribavirin. However, these regimens had low sustained virologic response (SVR) rates and were poorly tolerated. During the era of P-IFN therapy, several studies showed that the presence of obesity and/or steatosis led to a reduction of SVR rate in HCV patients. In patients with diabetes and HCV who were treated with IFN-based therapies, HCV clearance was associated with improved insulin resistance and beta cell function.

Research motivation

In 2013 and 2014, approval of newer direct acting antiviral agents (DAA) created IFN-free regimens with SVR rates greater than 90%, radically changing HCV treatment. Due to the novelty of DAA regimen, research is being actively pursued in a variety of patient populations.

Research objectives

We aim to determine if successful treatment with DAA is associated with improvements in hemoglobin A1c (HbA1c) and if the presence of T2DM or metabolic syndrome affects SVR rates.

Research methods

DAA were introduced to the VA Greater Los Angeles Healthcare System (VAGLAHS) at the beginning of April 2014. Therefore, we included all patients being treated with DAA between April 1st, 2014 and April 30th, 2016 for this study.

We performed a retrospective analysis of all HCV patients at the VA Greater Los Angeles Healthcare System treated with DAA therapy between 2014-2016. Separate multivariable logistic regression was performed to determine predictors of HbA1c decrease ≥ 0.5 after DAA treatment and predictors of SVR 12-wk post treatment (SVR12). Patients with HCV were also included if they had diabetic medications on their medication list during the study period. Patients were excluded from the cohort if they did not have SVR12 data or a HbA1c one year after completion of DAA therapy.

Research results

A total of 1068 patients were treated with DAA therapy between 2014-2016. The presence of T2DM or metabolic syndrome did not adversely affect SVR12. 106 patients had both HCV and T2DM. Within that cohort, patients who achieved SVR12 had lower mean HbA1c pre-treatment (7.35 vs 8.60, $P = 0.02$), and lower mean HbA1c post-treatment compared to non-responders (6.55 vs 8.61, $P = 0.01$). The mean reduction in HbA1c after treatment was greater for those who achieved SVR12 than for non-responders (0.79 vs 0.01, $P = 0.03$). In adjusted models, patients that achieved SVR12 were more likely to have a HbA1c decrease of > 0.5 than those that did not achieve SVR12 (adjusted OR = 7.24, 95%CI: 1.22-42.94).

Research conclusions

In conclusion, this study of a diverse VA patient population demonstrates that HCV clearance with DAAs is associated with a clinically significant decrease in HbA1c. This change was consistent across all genotypes and treatment regimens. The change in HbA1c was independent of changes in BMI and DM medication requirements and so may represent a decrease in host insulin resistance or increased insulin sensitivity. This study substantiates similar studies during the pegylated-interferon era by showing that HCV clearance irrespective of treatment regimen and genotype leads to improved DM outcomes. However, unlike prior studies during the period of pegylated-interferon, we show that hypertension, hyperlipidemia, T2DM, obesity, and metabolic syndrome do not negatively affect SVR12 rates for DAA.

Research perspectives

Future prospective studies analyzing patient fasting blood glucose and serum insulin should be performed to validate these findings and to help elucidate the relationship between HCV, insulin sensitivity, and longer-term DM outcomes such as cardiovascular events.

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Observational Study

Chronic hepatitis B virus monoinfection at a university hospital in Zambia

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Abstract

AIM

To characterize antiviral therapy eligibility among hepatitis B virus (HBV)-infected adults at a university hospital in Zambia.

METHODS

Hepatitis B surface antigen-positive adults ($n = 160$) who were HIV-negative and referred to the hospital after a routine or clinically-driven HBV test were enrolled. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), platelet count, hepatitis B e-antigen, and HBV DNA were measured. Liver fibrosis/cirrhosis was assessed by physical examination, AST-to-platelet ratio index, and transient elastography. In antiviral therapy-naïve individuals, we described HBV stages and antiviral therapy eligibility per World Health Organization (WHO) and by HBV test (routine *vs* clinical). Elevated ALT was > 19 in women and > 30 U/L in men. Among treatment-experienced individuals, we described medication side effects, adherence, and viral suppression.

RESULTS

The median age was 33 years, 71.9% were men, and 30.9% were diagnosed with HBV through a clinically-driven test with the remainder identified *via* routine testing (at the blood bank, community events, *etc.*). Among 120 treatment-naïve individuals, 2.5% were categorized as immune tolerant, 11.7% were immune active, 35.6% were inactive carriers, and 46.7% had an indeterminate phenotype. Per WHO guidelines, 13 (10.8%) were eligible for immediate antiviral therapy. The odds of eligibility were eight times higher for those diagnosed at clinical *vs* routine settings (adjusted odds ratio, 8.33; 95%CI: 2.26-29.41). Among 40 treatment-experienced HBV patients, virtually all took tenofovir, and a history of mild side effects was reported in 20%. Though reported adherence was good, 12 of 29 (41.4%) had HBV DNA > 20 IU/mL.

CONCLUSION

Approximately one in ten HBV-mono-infected Zambians were eligible for antivirals. Many had indeterminate phenotype and needed clinical follow-up.

Key words: Hepatitis B virus; Liver fibrosis; Treatment; Tenofovir; Africa

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Core tip: Data to inform the scale-up of hepatitis B testing and treatment in Africa are badly lacking. Among 120 recently diagnosed hepatitis B surface antigen-positive and HIV negative adults in Zambia, Southern Africa, 10% met the WHO's criteria for immediate antiviral therapy and an additional 40% had an "indeterminate" hepatitis B virus (HBV) phenotype with either elevated alanine aminotransferase or HBV DNA > 2000 IU/mL. Among 40 additional patients who were antiviral therapy-experienced (primarily with tenofovir), tolerability and adherence were good; however, nearly half had incomplete HBV DNA suppression. Effective approaches to retain antiviral-ineligible HBV patients in care will be important in Zambia.

Vinikoor MJ, Sinkala E, Kanunga A, Muchimba M, Nsokolo B, Chilengi R, Wandeler G, Mulenga J, Chisenga T, Bhattacharya D, Saag MS, Foster G, Fried MW, Kelly P. Chronic hepatitis B virus mono-infection at a university hospital in Zambia. *World J Hepatol* 2018; 10(9): 622-628 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v10/i9/622.htm> DOI: <http://dx.doi.org/10.4254/wjh.v10.i9.622>

INTRODUCTION

In Africa, approximately 60 million individuals are chronically infected with hepatitis B virus (HBV). Informed by Asian and European data, international recommendations were developed to guide policymakers and implementers on diagnosis and treatment of chronic HBV in resource-constrained settings^[1-3]. In settings with hepatitis B surface antigen (HBsAg) positivity $> 2\%$, both general population testing and targeted testing of populations with higher disease burden are recommended; however, limited real world data from Africa are available. In addition, understanding the proportion of patients diagnosed with chronic HBV infection who would benefit from antiviral therapy in African settings is not well-established.

Several recent studies have informed scale-up of HBV treatment in Africa. In Zambia, integration of HBsAg testing into a population-based HIV survey revealed 5.6% of adults and 1.3% of children to be HBsAg-positive (Zambian Ministry of Health, 2016 #599). A research program in Gambia demonstrated the feasibility of community screening, linkage to a comprehensive liver evaluation, and initiation of antiviral treatment^[4]. Among potential key populations (sex workers and men who have sex with men) in West Africa and adults in Ethiopia, approximately 10% met criteria for antiviral therapy but another 50% had markers requiring longitudinal follow-

up^[5]. Building on these and other studies, we established an observational cohort study at a referral hospital in Lusaka, Zambia. We described where Zambians are currently being tested for HBV and among those with no prior HBV treatment, we categorized patients into the classical stages of HBV using biochemical, virological, and non-invasive markers of liver fibrosis. We also estimated patient eligibility for antivirals according to international guidelines. Among patients already taking antiviral therapy, we described medication side effects, adherence, and viral suppression.

MATERIALS AND METHODS

Study setting and participant recruitment

In Zambia, 5.6% of adults and 1.3% of children are estimated to be HBsAg-positive^[6], and HBV genotypes A1 and E are present at nearly equal proportions^[7]. HBsAg testing is part of the evaluation of signs or symptoms of liver disease, and routine testing in Zambia occurs at several settings, including at the blood bank, during community screening events (sometimes integrated with HIV testing), and as part of medical check-ups to obtain a driving license, enroll in college, start a new job, *etc.* Following an HBsAg-positive diagnosis, some Lusaka area residents are referred to University Teaching Hospital (UTH), a public tertiary care and academic medical facility with > 1000 inpatient beds located in Zambia's capital of Lusaka. At UTH, the Department of Internal Medicine hosts a weekly liver clinic that is staffed by three gastroenterology-trained physicians and provides specialty care for patients with acute and chronic liver disease, hepatic lesions, and portal hypertension.

From August 23, 2016 to August 18, 2017, after obtaining the required ethical and government approvals, 160 HBsAg-positive HIV-negative adults (18+ years old) were enrolled in a cohort study at the UTH liver clinic. HIV-HBV coinfecting individuals identified during recruitment for the cohort were referred for immediate linkage to and initiation of antiretroviral therapy according to national guidelines^[8]. We excluded patients from provinces outside of Lusaka to reduce losses to follow-up. Participants provided written informed consent to be followed for up to five years.

Study measures

Although specific HBV management guidelines are limited in Zambia, international standards of care are followed. At cohort enrollment, we performed a complete physical examination, including vital signs and body mass index (BMI), and extracted data from participants' medical records related to HBV testing, treatment, laboratory and imaging tests, and liver biopsy results when available. A standardized questionnaire was used to document sociodemographic information, comorbid conditions, and to screen for and quantify alcohol consumption using the alcohol use disorders identification

test-consumption (AUDIT-C)^[9]. Among those already on antiviral drugs for HBV at cohort enrollment, we assessed current or prior drug side effects and self-reported medication adherence in the past 7 d.

Blood was collected for measurement of serum transaminases, hemoglobin, platelet count, hepatitis B e-antigen (HBeAg), and HBV viral load, which was determined using either the Roche COBAS AmpliPrep/COBAS Taqman platform (Pleasanton, CA, United States) or an in-house real time PCR assay^[10]. We measured liver stiffness non-invasively with the Aspartate Amino-transferase (AST)-to-platelet ratio index^[11] and with transient elastography (TE; Fibroscan 402, Echosens, Paris, France). TE was performed by a nurse or physician trained according to manufacturer guidelines and with experience performing > 500 tests. Testing for hepatitis C and hepatitis delta was not routinely performed as they are rare in Zambia^[12,13].

Statistical analysis

Among treatment-naïve patients (*i.e.*, no prior history of antiviral therapy), we described demographics and clinical features stratified by type of HBsAg test (clinical or routine). We compared baseline characteristics measured on categorical scale between clinical and routine diagnosis using Fisher's exact test. For comparison of median between the two groups, we used Wilcoxon rank sum test. Obesity was BMI > 30 and unhealthy alcohol consumption was AUDIT-C of 4+ for men and 3+ for women. Among antiviral naïve participants, we described HBV viral loads, HBeAg, serum transaminase levels, and the proportion of patients with cirrhosis. In our primary analysis, we defined elevated ALT as > 30 U/L for men and > 20 U/L for women per WHO recommendations^[3]. We defined persistently elevated ALT as any degree of baseline ALT elevation plus an elevated measurement at > 60 d prior to baseline. Significant fibrosis (equivalent to Metavir fibrosis stages F2-4) was defined as liver stiffness measurement (LSM) of 7.9-9.5 kPa based on a validation study in West Africa^[14]. We defined cirrhosis as having at least one of the following: APRI > 2.0^[11], LSM > 9.5 kPa^[14], or decompensated cirrhosis on physical examination defined by the presence of ascites.

Using ALT, HBeAg, and HBV DNA at enrollment, we categorized treatment-naïve participants into one of the classical stages of chronic HBV infection. Immune tolerant stage was defined as HBeAg-positivity, normal ALT, and HBV DNA > 20000 IU/mL. Immune active HBV was defined as either HBeAg-positive, elevated ALT, and HBV DNA > 20000 IU/mL or HBeAg-negative/unknown with elevated ALT and HBV DNA > 2000 IU/mL. Inactive carriers were those with HBeAg-negative/unknown status, normal ALT, and HBV DNA < 2000 IU/mL. Participants not categorized into one of these three stages were considered to have an indeterminate phenotype^[15]. We repeated this categorization using the "conventional" ALT upper limit of normal (*i.e.*, 40 IU/mL). We compared unhealthy alcohol use and obesity

between indeterminate patients with HBV DNA < 2000 and ALT elevation and inactive carriers using a χ^2 test.

We determined eligibility for antiviral therapy if patients met one of these criteria based on WHO guidelines: (1) decompensated cirrhosis; (2) APRI > 2.0; or (3) HBV DNA > 20000 IU/mL with ALT elevation and age > 30 years. We also assessed treatment eligibility per European Association for Study of the Liver (EASL) criteria^[2] as follows: (1) cirrhosis by physical examination (with or without decompensation) and detectable HBV DNA; (2) HBeAg-positive and age > 30 years; (3) LSM > 7.9 and HBV DNA > 2000 IU/mL, or (4) ALT > 80 U/L and HBV DNA > 20000 IU/mL. We compared treatment eligibility, per WHO criteria, between those who were diagnosed with a clinically-driven vs routine HBsAg test using logistic regression and adjusting for age and sex.

Among treatment experienced patients, we described antiviral therapies received, time on therapy, history of side effects, and self-reported adherence. We reported the proportion with viral suppression (VS) defined as HBV DNA < 20 IU/mL among those on therapy for 2+ years. Analyses were performed using Stata version 14 (Statacorp, College Station, TX, United States). The statistical review of the study was performed by a biomedical statistician. The cohort was approved by the ethics committees at University of Zambia (Lusaka, Zambia) and University of Alabama at Birmingham (Birmingham, AL, United States).

RESULTS

Median age was 33 years (IQR, 26-42), 115 (71.9%) were men, and the majority ($n = 84$, 52.6%) were recently diagnosed with HBV. The majority were diagnosed at routine HBsAg testing during community/routine medical check-ups ($n = 58$; 36.2%) or at the blood bank, while 49 (30.6%) were tested due to signs/symptoms of possible liver disease (*i.e.*, clinical test). Current alcohol consumption at "unhealthy levels" was reported by 19 (12.2%) participants. At enrollment, 120 (75.0%) were antiviral therapy naïve. Serum transaminases were available for 145 (90.6%) and 104 (65.0%) had a prior ALT measurement available at a median of 308 d (IQR, 62-469) before enrollment. HBeAg testing was performed for 143 (89.4%), 149 (93.1%) had an HBV DNA measurement, and 97 (60.6%) underwent TE. A description of treatment naïve patients by HBsAg test type (routine vs clinical) is shown in Table 1.

Among 120 treatment naïve patients, 5 (4.2%) had decompensated cirrhosis (*i.e.*, ascites) by physical examination. Among the 62 with sufficient data, median APRI was 0.29 (IQR, 0.18-0.51) and 4 (6.4%) had APRI > 2.0. Among the 69 that underwent TE, median LSM was 6.3 kPa (IQR, 4.8-8.3), 6 (8.7%) had LSM suggestive of significant fibrosis, and 14 (20.3%) had LSM suggestive of cirrhosis. A cumulative 17 (14.2%) patients had cirrhosis by either physical examination,

APRI, or TE. Median ALT was 23 (IQR, 17-36) and 44 (40.7%) had an elevated ALT at enrollment based on WHO-recommended thresholds. Among the 66 with serial ALT levels, 30 (45.4%) had persistently normal ALT, 20 (30.3%) had intermittently elevated ALT, and 16 (24.2%) had persistently elevated ALT. Median HBV DNA level was 232 IU/mL (IQR, 23-3495) and viral loads were low (*i.e.*, < 2000 IU/mL) for 77 patients (69.4%), moderate (2000-20000 IU/mL) for 16 (14.4%), and high (> 20000 IU/mL) for 18 (16.2%). HBeAg-positivity was present in 18 (18.9%), and among HBeAg-positives, 9 (50.0%) had high and 5 (27.8%) had moderate HBV DNA levels with 4 at HBV DNA < 2000 IU/mL.

Using baseline ALT, HBV DNA, and HBeAg, we categorized 3 (2.5%) treatment-naïve patients as immune tolerant, 14 (11.7%) as immune active, 47 (35.6%) as inactive carriers, and 56 (46.7%) as having indeterminate stage. While 18 of 56 were considered indeterminate due to a missing ALT, HBV DNA, or HBeAg, 29 had HBV DNA < 20000 IU/mL with elevated ALT and 7 had HBV DNA > 20000 with normal ALT. Elevated ALT in patients with indeterminate stage could not be attributed to overweight/obesity or unhealthy alcohol use, as rates of these were similar to those of inactive carriers (data not shown). After applying the conventional ALT threshold (*i.e.*, 40 IU/mL), there were 6 (5.0%) immune active patients, 10 (8.3%) immune active patients, 65 (54.2%) inactive carriers, and 39 (32.5%) with an indeterminate phenotype.

According to WHO guidelines, 13 (10.8%) treatment naïve patients were deemed to be eligible for antiviral therapy at cohort enrollment (Table 2). Of these, five became eligible for decompensated cirrhosis, two for APRI > 2.0, and six for HBV DNA > 20000 IU/mL with ALT elevation and age > 30 years. Among patients diagnosed during a routine HBsAg-test, 4 (4.6%) met WHO treatment criteria vs 9 (29.0%) of those with clinically-driven HBsAg testing. After adjusting for age and sex, there was 8 times higher odds of treatment eligibility for clinically vs routinely-diagnosed patients (adjusted odds ratio, 8.33; 95%CI: 2.26-29.41). By EASL guidelines, 21 (17.5%) were eligible for antiviral therapy.

Among 40 treatment-experienced patients, 31 were currently taking antivirals at enrollment with the majority (85%) on fixed dose combination TDF plus 3TC. Median time on therapy was 12.2 mo (IQR, 5.0-24.3). Although complete medical records were not available for all patients, many treatment-experienced patients had a history of significant fibrosis/cirrhosis at time of initiation. A minority of patients ($n = 6$; 20.0%) reported at least one side effect in the past/present attributed to antivirals, including nausea, diarrhea, skin rash, itchiness, dizziness, drowsiness, or mild headache. Among those on therapy at enrollment, 17 of 29 (58.6%) had complete HBV DNA suppression including 5 of 9 with 2+ years on treatment. Among the four individuals with HBV DNA non-suppression during long-term

Table 1 Baseline characteristics of treatment-naïve hepatitis B mono-infected adults referred to a university hospital in Zambia according to setting of hepatitis B virus diagnosis

	Clinical diagnosis (<i>n</i> = 31)	Routine diagnosis (<i>n</i> = 89)	<i>P</i> value
Median age, in years	37 (29-42)	33 (26-41)	0.15
Male sex	22 (71.0)	63 (70.8)	0.99
Education level completed			0.05 ^a
None to 6 th grade	5 (16.1)	3 (3.4)	
7 th to 12 th grade	14 (45.2)	49 (55.1)	
College	12 (38.7)	37 (41.6)	
Lifetime alcohol abstinence	13 (41.9)	36 (40.9)	0.92
Herbal medicine use, past month	7 (31.8)	14 (16.3)	0.10
Body mass index			
< 25	23 (76.7)	56 (62.9)	0.35
25-30	5 (16.7)	20 (22.5)	
> 30	2 (6.7)	13 (14.6)	
HBV DNA level, IU/mL			0.12
< 20	4 (15.4)	21 (24.7)	
20-1999	10 (38.5)	42 (49.4)	
2000-19199	4 (15.4)	12 (14.1)	
≥ 20000	8 (30.8)	10 (11.8)	
HBeAg positive	11 (37.9)	11 (14.5)	0.008 ¹
Median ALT, in U/L (IQR)	22 (17-28)	23 (17-36)	0.82
Elevated ALT	8 (32.0)	36 (43.4)	0.31
AST-to-platelet ratio index ≥ 2.0	4 (21.1)	0	0.002 ¹
Liver stiffness measurement in kPa			
< 7.9	4 (25.0)	45 (84.9)	< 0.001 ¹
7.9-9.5	2 (12.5)	4 (7.6)	
> 9.5	10 (62.5)	4 (7.6)	

¹Data with statistical significance. HBV: Hepatitis B virus; ALT: Alanine aminotransferase. All values are *n* (%) or median (IQR).

therapy, all HBV DNA levels were < 2000 IU/mL. Among those currently on antivirals, median adherence was 100% but 12.5% reported missing at least one dose in the prior 7 d.

DISCUSSION

Approximately 1 in 10 Zambian adults with HBV mono-infection met international guidelines for immediate antiviral therapy, and an additional half had either elevated ALT or HBV DNA > 2000 IU/mL, suggesting the need for further follow-up. Participants diagnosed on suspicion of having HBV were more likely to require therapy compared to those HBsAg tested at routine settings. Antiviral therapy was well tolerated among treatment-experienced participants; however, a subset had HBV DNA non-suppression. These data are some of the first data on modern HBV treatment in Southern Africa and provide insights around the scale-up of testing for and treatment of chronic HBV infection in Africa.

While many chronic HBV patients in Zambia had elevated ALT, relatively few qualified for immediate antiviral therapy when we applied international guidelines. By WHO guidelines around 10% of treatment-naïve patients met criteria and by EASL criteria this was closer to 20%. These results are supported by Gambian data where 4.4% in the community and 9.7% of blood donors met EASL criteria^[4]. In a smaller study of female sex workers, men who have sex with men, and inmates

in West Africa, 10.0% were also treatment-eligible based on modified WHO criteria^[5]. Taken together these research data suggest that the WHO criteria are very stringent and might be missing some patients who could benefit from antiviral therapy. Although not surprising, we also observed that symptomatic patients were more likely to meet treatment eligibility compared to those tested in routine settings. Further data are needed to understand the most efficient way to identify HBV patients who would benefit from antiviral therapy in Zambia and similar settings.

Based on their enrollment values, 35% of our participants were classified as inactive carriers and half had indeterminate HBV phenotypes. Identification of inactive carriers, a group at low risk to progress to cirrhosis or develop HCC^[16], is useful in programmatic settings in Africa. For example, at an HBV treatment program in Ethiopia, inactive carriers (also representing around one-third of patients) will be discharged from care after one year in order to focus resources on those more likely to benefit from therapy^[17]. Unfortunately, our cohort and others have reported that a substantial percentage of patients cannot be initially categorized as needing or not needing therapy (*i.e.*, indeterminate phenotype). In Senegal, 53% did not qualify for antivirals but had either significant fibrosis, raised ALT, or significant HBV DNA levels^[5]. In the HBV Research Network (HBRN) in North America, 38% had indeterminate phenotype at baseline and will be followed longitudinally^[15]. Similar to the HBRN, most patients with indeterminate disease stage

Table 2 Hepatitis B virus stage and eligibility for immediate antiviral therapy among treatment-naïve Zambian adults with chronic hepatitis B virus infection *n* (%)

		Overall (<i>n</i> = 120)	Clinical diagnosis (<i>n</i> = 31)	Routine diagnosis ¹ (<i>n</i> = 89)	<i>P</i> ²
HBV stage	Immune tolerant	3 (2.5)	2 (6.4)	1 (1.1)	0.16
	Immune active	14 (11.7)	4 (12.9)	10 (11.2)	0.8
	Inactive carrier	47 (35.6)	7 (20.0)	40 (41.2)	0.02 ³
	Indeterminate	56 (46.7)	18 (58.1)	38 (42.7)	0.14
Eligibility for immediate therapy per guidelines	WHO 2015 guidelines	13 (10.8)	9 (29.0)	4 (4.5)	< 0.01 ³
	EASL 2017 guidelines	21 (17.5)	12 (38.7)	9 (10.1)	< 0.01 ³

¹Routine diagnosis was defined as being tested for hepatitis B surface antigen at the blood bank, antenatal care, a community screening event, or as part of a routine medical check-up; ²A χ^2 test was used to compare clinically and routinely HBV-diagnosed participants; ³Data with statistical significance. HBV: Hepatitis B virus; WHO: World Health Organization; EASL: European Association for Study of the Liver.

in Zambia had elevated ALT with HBV DNA < 20000 IU/mL, suggesting non-HBV reasons for ALT elevation. Although we did not find that they were correlated with having indeterminate stage, fatty liver and hazardous alcohol use are potential causes of elevated ALT in HBV patients. Longitudinal follow-up to ascertain whether patients of indeterminate stage develop treatment indications or become inactive carriers is needed in Africa to guide HBV policy^[5].

Our data from treatment-experienced Zambians with chronic HBV monoinfection support the feasibility of longitudinal treatment with antiviral therapy in African settings. TDF + XTC was the most common regimen as this fixed-dose combination drug is found in large supply in Zambia to treat and prevent HIV/AIDS. We documented HBV DNA non-suppression among the small group on long-term therapy which requires further follow-up. We presumed suboptimal adherence as the mechanism, although most of our patients self-reported good adherence in the prior week. Our data are contrasted by the PROLIFICA study in the Gambia where 43 of 47 (91.5%) tenofovir-treated HBV mono-infected individuals achieved viral suppression at one year^[4]. Lower HBV DNA suppression in our cohort may reflect differences in study design (clinical trial vs hospital cohort) or could reflect HBV genotypic differences as Gambia has predominantly HBV genotype E and Zambia has both E and A1, a genotype that rapidly developed lamivudine-resistance in Malawi^[18].

This study has several limitations. Most importantly, our cohort is based at one site in Zambia and may not represent other HBV-infected populations. Our cohort is hospital-based and enriched for sicker patients evidenced by the fact that participants referred from clinical settings were eight times more likely to be treatment-eligible compared to other HBsAg-positives. To offset this, we also characterized a subset of participants diagnosed at routine settings such as the blood bank and a population-based survey. Finally, we had incomplete laboratory and clinical data that led to incomplete evaluation of some patients and inflated our estimate of the proportion with indeterminate HBV. We believe the missing data occurred at random and would not have introduced bias into the distribution of HBV stages.

In summary, an HBV monoinfection cohort was established at a referral hospital in Zambia to begin to answer a number of clinical and operational questions around HBV treatment in Africa. We observed that 1 in 10 patients who underwent comprehensive assessment met the WHO criteria for therapy, although the number was higher among those with signs/symptoms and lower among those diagnosed during routine HBsAg testing. These data support scale-up of HBV testing and treatment in Africa, but further operational and clinical research is needed to define the most effective and efficient way to reduce HBV-related mortality and morbidity.

ARTICLE HIGHLIGHTS

Research background

Africa has 60 million individuals living with chronic hepatitis B virus (HBV) infection yet limited data to inform how to identify, link to care, and treat them to reduce the burden of cirrhosis and liver cancer.

Research motivation

Not all HBV patients need antivirals. Estimates on how many do will guide policy implementation. Also, few data are available on patient adherence, retention, and viral suppression with current antiviral drugs.

Research objectives

Our objective was to perform a comprehensive clinical assessment on chronic HBV-infected adults in Zambia and apply international criteria to learn what percentage may need antiviral drugs. In those already on antivirals, we measured the viral control.

Research methods

At a university hospital in Zambia, a cross-sectional assessment of adults (18+ years old) who were hepatitis B surface antigen positive and HIV negative was undertaken during 2016-2017. We used tests available in upper-income settings such as HBV DNA testing and transient elastography to assess HBV in these patients.

Research results

One hundred and sixty enrolled in the study, including 120 who were recently diagnosed with HBV and 40 already on antiviral drugs. The average age was 33, and 72% were men. We found that 1 in 10 met the World Health Organization guidelines to start antivirals; however, nearly 1 in 2 had at least one finding that would need clinical follow-up. Patients diagnosed because of signs or symptoms of HBV were slightly more likely to need antivirals compared to those diagnosed via routine testing (such as at the blood bank). Among those

already on the antivirals, few had side effects; however, 41% did not completely control their viral load.

Research conclusions

This is the first Southern African study to apply international HBV criteria.

Research perspectives

Additional data are needed on whether those with high ALT or viral loads at baseline will later need antivirals. HBV testing that focuses on symptomatic individuals could be more efficient (than routine testing for all) to find those needing treatment but more information is needed.

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Acute liver failure secondary to severe systemic disease from fatal hemophagocytic lymphohistiocytosis: Case report and systematic literature review

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Abstract

AIM

To systematically review liver disease associated with hemophagocytic lymphohistiocytosis (HLH), propose reasonable contraindications for liver transplantation for liver failure in HLH, and report an illustrative case.

METHODS

Systematic review according to PRISMA guidelines of hepatic manifestations of HLH using computerized

literature search *via* PubMed of articles published since 1980 with keywords ("hemophagocytic lymphohistiocytosis" or "HLH") AND ("liver" or "hepatic"). Two authors independently performed literature search and incorporated articles into this review by consensus. Illustrative case report presented based on review of medical chart, and expert re-review of endoscopic photographs, radiologic images, and pathologic slides.

RESULTS

A 47-year-old Caucasian male, was hospitalized with high-grade pyrexia, rash, total bilirubin = 45 g/dL, moderately elevated hepatic transaminases, ferritin of 3300 ng/dL, leukopenia, and profound neutropenia (absolute neutrophil count < 100 cells/mm³). Viral serologies for hepatitis A, B, and C were negative. Abdominal computed tomography scan and magnetic resonance imaging revealed no hepatic or biliary abnormalities. Pathologic analysis of liver biopsy revealed relatively well-preserved hepatic parenchyma without lymphocytic infiltrates or macrophage invasion, except for sparse, focal hepatocyte necrosis. Bone marrow biopsy and aspirate revealed foamy macrophages engulfing mature and precursor erythrocytes, consistent with HLH. Interleukin-2 receptor (CD25) was highly elevated, confirming diagnosis of HLH according to Histiocytic Society criteria. Patient initially improved after high-dose prednisone therapy. Patient was judged not to be a liver transplant candidate despite model for end stage liver disease (MELD) score = 33 because liver failure was secondary to severe systemic disease from HLH, including septic shock, focal centrilobular hepatocyte necrosis from hypotension, bone marrow failure, and explosive immune activation from HLH. The patient eventually succumbed to overwhelming sepsis, progressive liver failure, and disseminated intravascular coagulopathy. Systematic review reveals liver injury is very common in HLH, and liver failure can sometimes occur. Data on liver transplantation for patients with HLH are very limited, and so far the results have shown a generally much worse prognosis than for other liver transplant indications. Liver transplantation should not be guided solely by MELD score, but should include liver biopsy results and determination whether liver failure is from intrinsic liver injury *vs* multisystem (extrahepatic) organ failure from HLH.

CONCLUSION

This case report illustrates that liver transplantation may not be warranted when liver failure associated with HLH is primarily from multisystem failure from HLH. Liver biopsy may be very helpful in determining the severity and pathophysiology of the liver disease.

Key words: Hemophagocytic lymphohistiocytosis; Acute liver failure; Liver injury; Liver transplantation; Acquired immune hyperactivation; Pancytopenia

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Core tip: This work systematically reviews liver disease in hemophagocytic lymphohistiocytosis (HLH), proposes contraindications for liver-transplantation in such patients, and reports an illustrative case. A 47-year-old-man was hospitalized with high-grade pyrexia, total-bilirubin = 45 g/dL, and profound neutropenia. Bone marrow biopsy/aspirate revealed hemophagocytic histiocytes. The patient had HLH, satisfying 6 Histiocytic Society criteria. Liver-biopsy histopathology revealed relatively well-preserved hepatic parenchyma, except for sparse hepatocytic necrosis. The patient was not a liver-transplant-candidate, despite model for end stage liver disease-score = 33, due to multi-organ failure from HLH. He expired from multi-organ failure despite receiving corticosteroids. This case and systematic review reveals patients with HLH may not be liver transplant candidates because of liver failure from multisystem failure.

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INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of aggressive immune hyperactivation from hypercytokinemia that frequently causes liver injury and sometimes causes acute liver failure (ALF) which can contribute to the high syndromic mortality. Systematic literature review revealed hundreds of reported cases of HLH in adults, but provides insufficient data on indications and contraindications for liver transplantation for ALF associated with HLH^[1]. Liver transplantation for ALF associated with HLH is currently controversial due to prominent systemic morbidities from HLH, the generally poor condition of patients suffering from both ALF and HLH, potential curability of ALF from HLH with HLH-specific therapy alone, and risk of recurrent HLH after liver transplantation^[2,3]. A case is reported of fatal HLH in an adult presenting prominently with ALF, with the liver injury primarily due to severe systemic disease from HLH, as documented by liver biopsy and clinical evaluation, and liver transplantation was refused on this basis. This case report is important in illustrating potential pitfalls in liver transplantation for ALF associated with HLH.

MATERIALS AND METHODS

This case was thoroughly reviewed based on medical chart, including re-review of original endoscopic photographs by an expert endoscopist, radiologic images by an expert radiologist, and pathologic slides by an

expert pathologist. The IRB at William Beaumont Hospital, Royal Oak exempted/approved this case report on November 15, 2017.

Literature on hepatic manifestations of HLH was systematically reviewed searching PubMed for articles with the following medical subject headings/keywords ("hemophagocytic lymphohistiocytosis" or "HLH") AND ("liver" or hepatic"), and by reviewing sections on HLH in standard pathology textbooks/monographs. Articles before 1980 were selectively excluded because clinical evaluations before 1980 often lacked currently required clinical tests. Large clinical trials, meta-analyses, systematic reviews, and controlled trials were assigned higher priority than review articles or small clinical series, and individual case reports were assigned the lowest priority. Two authors independently performed a literature search, and decided on which articles to incorporate in this review according to article priority based on consensus. Dr. Cappell has considerable experience in conducting systematic reviews, with 4 published systematic reviews in peer-reviewed journals indexed in PubMed during the last 2 years, and with a PhD in neurophysiology that involved 5 years of training and research in biomedical statistics.

Illustrative case report

A 47-year-old, non-alcoholic, non-obese, Caucasian man with type II diabetes mellitus treated with oral glipizide and metformin for 4 years, and no prior liver disease presented with a pruritic, maculopapular rash over his entire body associated with progressive fatigue, pyrexia, and profound jaundice for 7 d that began just after completing a 10-d course of oral trimethoprim-sulfamethoxazole therapy for an upper respiratory infection. Physical examination on admission revealed temperature = 39.5 °C, blood pressure = 89/48 mmHg, profound jaundice, no abdominal tenderness, no hepatosplenomegaly, no peripheral stigmata of chronic liver disease, and no peripheral lymphadenopathy. Laboratory values included: Leukocyte count = 700/μL (lab normal: 3500-10100/μL), and platelets = 283000/μL (normal: 150000-400000/μL). The sodium = 116 mmol/L (normal: 135-145 mmol/L), and creatinine = 1.67 mg/dL (normal: 0.60-1.40 mg/dL). Total bilirubin = 45.1 mg/dL (normal: 0.3-1.2 mg/dL), direct bilirubin = 26.4 mg/dL (normal: 0.0-0.3 mg/dL), alkaline phosphatase = 490 U/L (normal: 30-110 U/L), aspartate aminotransferase (AST) = 70 U/L (normal: 10-37 U/L), alanine aminotransferase (ALT) = 167 U/L (normal: 9-47 U/L), gamma glutamyl transferase = 730 U/L (normal: 13-60 U/L), albumin = 2.4 g/dL (normal: 3.5-5.1 g/dL), international normalized ratio (INR) = 1.9 (normal: 0.9-1.1), and lipase = 9 U/L (normal: 7-60 U/L). The cholesterol = 661 mg/dL (normal: 70-199 mg/dL), triglyceride = 184 mg/dL (normal: 30-149 mg/dL), and HDL cholesterol = 5 mg/dL (normal: 40-90 mg/dL). Serum IgG level was normal.

The acetaminophen level was 0. Acute viral hepatitis

panel was negative for hepatitis A, B, C, and E. Anti-smooth muscle and anti-mitochondrial antibodies were absent in serum. Genotyping for hemochromatosis was negative for C282Y, and was heterozygous for H63D mutation. Abdominal ultrasound and computerized tomographic scans revealed normal hepatobiliary findings. Magnetic resonance cholangiopancreatography revealed a normal biliary tree, and mild, diffuse, hepatosplenic iron deposition. Histopathologic examination of a transjugular liver biopsy revealed relatively well-preserved hepatic parenchyma without lymphocytic infiltrates or macrophages in nearly all the cores (Figure 1A and B), except for sparsely distributed, focal, and small centrilobular hepatocellular necrosis (Figure 1D and E). Although M30 and M65 serum levels were not determined as assays for apoptosis, the patient only had aggregates of nonviable hepatocytes on liver biopsy examination which strongly favors the diagnosis of hepatocellular necrosis over that of apoptosis. Immunohistochemical staining for CD-68, a marker for Kupffer cells and tissue macrophages, revealed only normal-appearing CD-68-positive Kupffer cells (dark brown staining, black arrows) with characteristic slender, elongated morphology in non-ischemic areas (Figure 1C); but revealed an additional population of plump, CD-68 positive macrophages (blue arrows) within areas of focal hepatic necrosis (Figure 1F). CD-71, a marker for nucleated erythrocytes, showed no nucleated erythrocytes (no hemophagocytosis) within the Kupffer cells in the non-ischemic area or within macrophages in the ischemic areas (negative immunostains not shown). Due to his employment as a sewer inspector, leptospirosis was excluded by absence of leptospiral antibodies. He was treated with intravenous (IV) aztreonam and vancomycin after isolating *Pseudomonas* and group A streptococcus from blood cultures.

Histopathologic examination of a bone marrow biopsy and aspiration smears revealed hypocellular bone marrow due to marked granulocytic hypoplasia and prominent histiocytic hemophagocytosis (Figure 2). Plasma cells present in the marrow showed no light chain restriction, and appeared to be reactive. Concomitant flow cytometry showed no evidence of Hodgkin's lymphoma or leukemia. The patient was diagnosed with HLH by satisfying the following 6 of 8 diagnostic criteria established by the Histiocyte Society (minimum 5 criteria required for diagnosis of HLH): (1) temperature = 39.5°C (qualifying-criterion: temperature > 38.5°C); (2) cytopenia of 2 blood cell lineages: hemoglobin = 8.6 g/dL (qualifying-criterion: hemoglobin < 9.0 g/dL, lab normal: 13.5-17.0 g/dL), and absolute neutrophil count < 100/μL (qualifying-criterion: < 1000/μL, lab-normal: 1600-7200 cells/μL); (3) fibrinogen = 135 mg/dL (qualifying criterion < 150 mg/dL; lab-normal: 175-375 mg/dL); (4) hemophagocytosis in bone marrow biopsy (qualifying-criterion: present); (5) ferritin = 3100 ng/mL (qualifying criterion: > 500 ng/mL, lab-normal: 14-338 ng/mL); and (6) elevated soluble interleukin 2

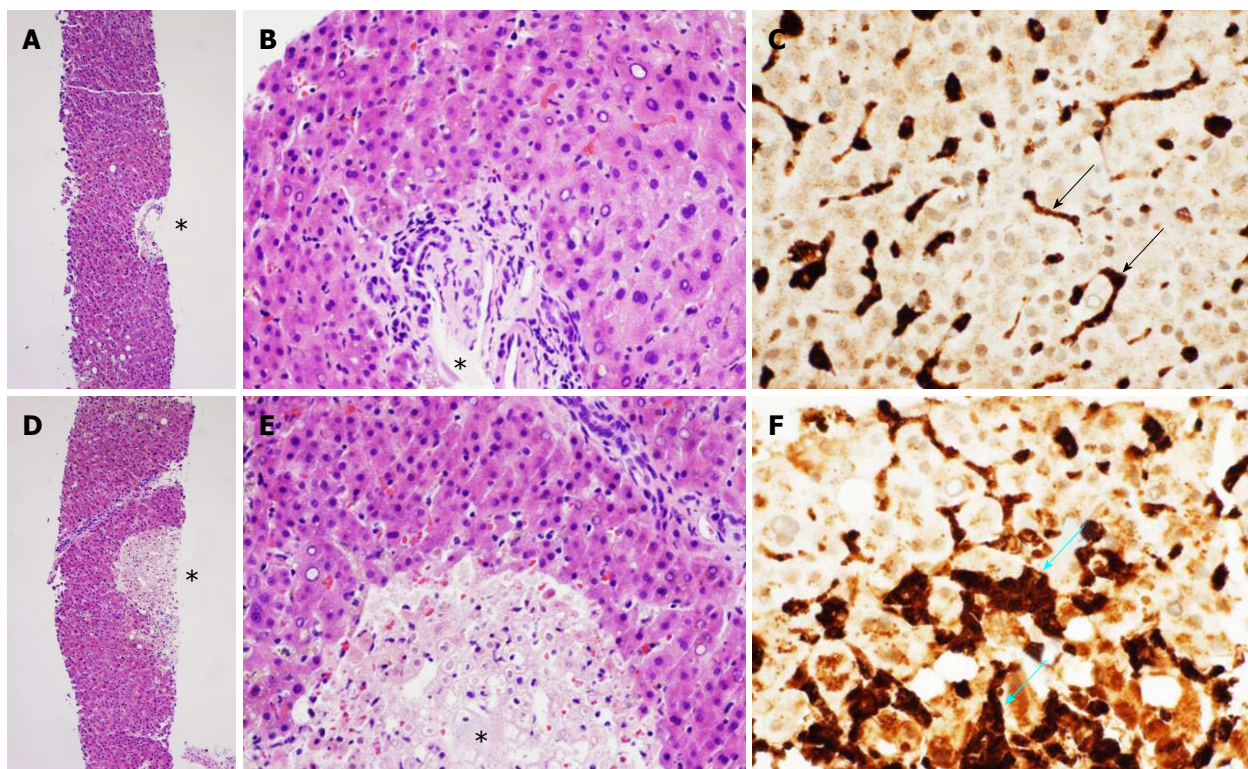


Figure 1 Liver biopsy. A, B: Low-power (A) and high-power (B) photomicrographs of a representative area of hematoxylin and eosin-stained section of the transjugular liver needle biopsy shows a normal portal triad (asterisk) and viable and relatively normal appearing hepatocytes with characteristically deeply eosinophilic cytoplasm and without evidence of hepatocyte necrosis, lymphocytic infiltrates, macrophages or hemophagocytic macrophages/histiocytes; C: Medium power photomicrograph with immunohistochemistry for CD-68, a molecular marker for Kupffer cells and tissue macrophages, shows CD-68 positive (brown staining) cells with elongated and slender morphology characteristic of Kupffer cells (arrows) within hepatic sinusoids; D, E: Low-power (D) and high-power (E) photomicrographs of hematoxylin and eosin-stained section of liver biopsy exhibiting focal centrilobular necrosis (asterisk), seen as a pale, circumscribed area containing pyknotic nuclei in liver biopsy specimen. Despite the liver failure, the areas of necrosis were sparsely distributed, and the overwhelming majority of the liver biopsy specimen contained viable and relatively well-preserved hepatocytes except for the cholestasis, demonstrated by a faint yellowish-brown tint in hepatocytes. No lymphocytic infiltrates or hemophagocytic macrophages/histiocytes are detected in this H and E stain; F: Medium-power photomicrograph with immunohistochemistry for CD-68, a molecular marker for Kupffer cells and macrophages, shows CD-68-positive (brown staining) plump cells with irregular contours characteristic of macrophages (blue arrows). The macrophages have been recruited to phagocytose the dying hepatocytes in the focally ischemic centrilobular area.

(IL-2)-receptor alpha = 9910 pg/mL (normal < 1033 pg/mL) (additionally, Histiocytic Society criterion #2-not satisfied, and criterion #6-not tested). Patient then received prednisone 100 mg orally daily. Liver transplant evaluation concluded that the patient was not a liver transplant candidate despite MELD score = 33 because of severe systemic illnesses. The patient and family refused experimental therapy with artificial hepatic assist devices, such as liver dialysis machines. Patient was not treated with porous CytoSorb polymer beads (CytoSorbents Corporation, Monmouth Junction, New Jersey, United States) to adsorb toxic molecules from septic shock because of no institutional experience with this treatment.

Patient then bled at the prior liver biopsy site because of disseminated intravascular coagulopathy. He became hypotensive, and was endotracheally intubated. Hemostasis was achieved by embolizing two major hepatic artery branches during two arteriogram sessions. He was transfused 13 units of packed erythrocytes, 6 units of fresh-frozen-plasma, 4 units of cryoprecipitate, and 4 units of platelets during this bleeding episode.

During the ensuing 10 d his ferritin increased > 33000 ng/mL, and the bilirubin and transaminase levels

modestly declined. Despite temporary clinical stabilization after endotracheal intubation, numerous antibiotic therapeutic regimens, IV fluids, and IV vasopressors, the patient succumbed to overwhelming sepsis, disseminated intravascular coagulopathy, and liver failure 42-d after admission.

RESULTS

HLH has no racial or sexual predilection^[4]. It most commonly affects infants < 18 mo old, but it can occur in older children and rarely occur in adults. It often presents with nonspecific symptoms and signs^[4-6].

HLH has primary (genetic) etiologies, which usually manifest in infants with genetic immunologic mutations^[7], and secondary (acquired) etiologies which typically manifest in older children or adults. Secondary etiologies include infection, especially with Epstein-Barr virus^[8,9] or herpes simplex virus-1^[10]; malignancy; or autoimmune diseases (called macrophage activation syndrome when HLH is associated with autoimmune diseases)^[11]. The common denominator in all acquired etiologies is disrupted immune homeostasis.

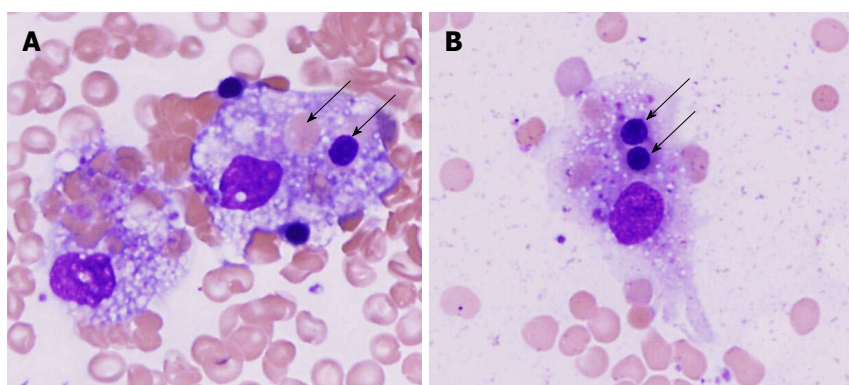


Figure 2 Hemophagocytosis in bone marrow. A: High-power photomicrograph of Diff Quick stain of bone marrow smear showing two large macrophages with foamy cytoplasm within a background of mature erythrocytes. The macrophage on the right has phagocytosed a mature erythrocyte (pink cell with no nucleus, higher arrow) and a nucleated erythrocyte precursor (pink cell with deeply purple nucleus, lower arrow); B: high-power photomicrograph of another large macrophage with foamy cytoplasm containing two phagocytosed nucleated erythrocyte precursor cells (purple nucleus, two arrows).

Prompt diagnosis is critical for patient survival, but the diagnosis is often delayed or overlooked, which contributes to its high mortality. HLH occurs in about 1% of hematologic malignancies^[12,13], sometimes from chemotherapy for these malignancies^[14]. Patients with malignancy have extremely high mortality, partly due to delayed diagnosis of HLH^[15,16]. Suggested mechanisms of HLH associated with malignancy include: profound inflammation from immune activation, persistent antigen stimulation by cancer cells, and deranged immune response secondary to chemotherapy.

The Histiocyte Society criteria, which are the generally accepted diagnostic criteria, are very strict and can, therefore, miss some cases of HLH (false negatives). HLH should be clinically considered in patients satisfying 3 or 4 criteria^[4]. Moreover, the Histiocyte Society incorporates two tests with limited clinical availability: Level of α chain of soluble interleukin-2 receptor (sIL-2r)^[17], and 51-Cr release assay reflecting NK-cell activity; unavailability of these tests militate against early diagnosis and therapy. Ferritin levels provide a reliable prognostic indicator and monitor of treatment efficacy^[18].

Treatment focuses on controlling the triggering event, such as underlying viral infection, when identified, and on 8-wk-induction-therapy with immunosuppressants, including corticosteroids, etoposide, and cyclosporine, to suppress exaggerated immune responses in HLH. The precipitating event in the current patient was likely the antecedent upper respiratory infection, but the specific (presumably viral) infectious agent was not identified despite extensive (viral) work-up. This patient was treated with high-dose prednisone, but not etoposide or cyclosporine because of the profound hyperbilirubinemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT), can sometimes achieve complete remission when the patient presents with profound HLH without life-threatening liver failure^[19-21].

Patients with established HLH commonly demonstrate markedly elevated biochemical parameters of liver function. For example, among 30 adult patients with HLH, about 60% had AST > 2 times the upper

limit of normal^[22,23]. Likewise, about 60% of patients had lactate dehydrogenase (LDH) > 2 times the upper limit of normal^[22,24]. About 80% of patients present with jaundice^[23], which is often profound^[25]. The hyperbilirubinemia is predominantly direct. In a study of 28 patients, 9 had moderately severe hypertriglyceridemia, with levels > 265 mg/dL, from cholestasis^[26]. Hypoalbuminemia is common, reflecting decreased liver synthetic function^[27]. Up to 90% of patients with advanced HLH have severe coagulopathies or disseminated intravascular coagulopathy^[22,23,28]. Liver injury is associated with overproduction of cytokines^[17]. Severely elevated ferritin levels, and iron overload, detected by hepatic magnetic resonance imaging (MRI), reflect severe hepatic injury.

Lymphocytes commonly infiltrate the liver from immune activation from HLH. For example, in a study of 27 autopsies of infants or children with congenital HLH, 22 (81%) had lymphocytic infiltrates, including 13 (48%) with dense lymphocytic infiltrates^[27]. Lymphocytic infiltrates are more commonly periportal than centrilobular^[28]. Treatment with corticosteroids or other immunosuppressants may decrease the density and distribution of intrahepatic lymphocytic infiltrates^[27]. Macrophages are occasionally present in the liver, but hemophagocytic macrophages are detected in only about 10% of liver biopsies, whereas hemophagocytic macrophages are commonly detected in spleen or bone marrow biopsies^[27].

ALF is currently reported secondary to HLH which was diagnosed based on satisfying 6 of 8 criteria for HLH. Despite biochemical evidence of severe hepatic injury, this patient had only focal hepatic necrosis, and relatively well-preserved hepatocytes in the rest of the biopsy sample, without intrahepatic lymphocytic infiltration or hemophagocytic macrophages. This patient had not received corticosteroids or other immunosuppressants prior to liver biopsy, which could have attenuated intrahepatic lymphocytic infiltration. These relatively bland liver biopsy findings in the setting of ALF strongly suggest that liver failure in this patient

with HLH is not necessarily from intrinsic liver disease, say from viral infection or drug toxicity, but may be secondary to multi-organ, systemic, and extrahepatic injury from HLH.

Liver transplantation has been offered to patients who present predominantly with ALF, from severe hepatic inflammation and necrosis, in association with potentially reversible HLH^[1-3]. In a literature review of 7 individual case reports, only 2 (28%) of 7 patients undergoing liver transplantation for ALF with HLH survived > 6 mo^[2,3]. In a small clinical series, 9 pediatric patients underwent liver transplantation for life-threatening ALF associated with secondary HLH^[3]. These patients typically had extremely abnormal liver function tests: ALT = 2512 ± 1158 U/L, (range: 1135-4113 U/L), AST = 3165 ± 2276 U/L (range: 779-8789 U/L), conjugated bilirubin = 257 ± 108 micromol/L (range: 141-448 micromol/L, normal 0-2 micromol/L), and INR = 7.7 ± 1.7 units (range: 3.9- > 9 units). Liver biopsy in these patients generally revealed severe-to-massive (25%-95%) hepatocyte necrosis, lymphocytic infiltrates, numerous macrophages, and intrahepatic erythrophagocytosis^[3]. These patients had extremely frequent medical and surgical complications after transplantation, including: severe or opportunistic infections-6, acute liver rejection-5, recurrent HLH-5, bile duct strictures-3, post-transplant lymphoproliferative disease of liver-2, bowel obstruction-1, and wound dehiscence-1. Three patients died at 1.5, 8, and 14 mo after liver transplantation. The other 6 pediatric patients were alive and well at a median of 24 mo after liver transplantation^[3].

In contradistinction, the currently reported patient had much less evident liver injury, but severe clinical manifestations of HLH. The current work may suggest that liver transplantation is not indicated for HLH when: (1)-ALF is not present or imminent (MELD score < 20-22); (2)-the patient has a poor prognosis due to the combined effects of ALF and HLH; (3)-the liver injury by itself does not underlie this poor patient prognosis; or (4)-the HLH is advanced and highly likely irreversible. This case illustrates three of these factors that militated against liver transplantation; in the current case the ALF was most likely from septic shock with focal centrilobular ischemia from hypotension, bone marrow failure, and explosive immune activation from HLH.

This prior clinical data suggest caution in liver transplantation for HLH. The data are limited. Reported outcomes for liver transplantation with HLH are much worse than that for liver transplantation without HLH^[3]. The current work suggests that high mortality from advanced and likely irreversible HLH may limit the benefits of liver transplantation. Liver biopsy may be very helpful to assess the liver injury and to determine whether ALF associated with HLH is due primarily to intrinsic liver injury vs secondary to extrahepatic injury from HLH to help determine whether liver transplantation is warranted.

About 25% of patients had a rash associated with HLH in one large study^[28]. The rash is most commonly generalized, maculopapular, and erythematous^[29]. Patients can also have petechiae and purpura from thrombocytopenia. This patient's rash started after administering trimethoprim-sulfamethoxazole and was most likely secondary to it.

DISCUSSION

HLH frequently involves the liver. It frequently causes AST levels > 2 times the upper limit of normal^[22,23], frequently causes LDH levels > 2 times the upper limit of normal^[22,24], and very frequently causes profound direct hyperbilirubinemia^[23,25]. Patients may also commonly present with moderately severe hypertriglyceridemia from cholestasis^[26], and hypoalbuminemia from decreased liver synthetic function^[27]. The liver injury is associated with overproduction of cytokines^[17], severe hepatic inflammation, and potentially hepatic necrosis.

When patients present with imminent or confirmed liver failure from predominantly liver involvement from HLH, liver transplant may be considered^[1-3]. In such cases, the liver failure may be documented by a MELD score > 24-26, and the liver failure should contribute significantly to the poor patient prognosis. Liver biopsy should be performed to determine the extent of the liver injury and the relative roles of intrinsic liver injury vs systemic HLH in the patient's ALF. However, the current case illustrates that patients may be poor candidates for liver transplantation from ALF secondary to HLH, if the HLH is likely irreversible, or if the patient has major comorbidities (systemic complications) from the HLH that are highly lethal. For example, the currently reported patient had highly lethal, comorbidities of overwhelming sepsis, shock, and disseminated intravascular coagulopathy, that precluded liver transplantation despite having a MELD score of 33 that would normally qualify the patient for liver transplantation. This work illustrates that decisions on liver transplantation in patients with HLH depend upon the presence of imminent or present ALF, the potential reversibility of the HLH, and the absence of severe comorbidities associated with the HLH that are most likely fatal. Patients with high MELD scores in the setting of HLH should be evaluated for liver transplantation before these highly lethal complications supervene.

Study limitations include: (1) this is a single retrospectively reported case report; (2) the etiology of HLH was undetermined, as occurs in about 25% of adult cases^[4,30]; (3) the microbiology of the antecedent upper respiratory infection, that likely stimulated the HLH, is unknown; (4) the reported pathologic findings at liver biopsy are subject to sample bias; and (5) the possible, but unlikely, role of trimethoprim-sulfamethoxazole in the ALF^[31]. Study strengths include the well-established diagnosis of HLH, and the academic evaluation of this patient at a tertiary-care-university hospital with a liver

transplant center.

ARTICLE HIGHLIGHTS

Research background

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of aggressive immune hyperactivation from hypercytokinemia that frequently causes liver injury and sometimes causes acute liver failure (ALF) that can contribute to the high syndromic mortality. Systematic literature review revealed hundreds of reported cases of HLH in adults, but provided insufficient data on the patterns and pathophysiology of the liver injury. It is particularly important to clinically determine whether ALF associated with HLH is due primarily to intrinsic liver injury vs secondary to extrahepatic causes from the HLH, to help determine whether liver transplantation is potentially warranted. A case is reported of fatal HLH in an adult presenting prominently with ALF, with the liver injury secondary to severe systemic disease from HLH, as documented by liver biopsy and clinical evaluation, and liver transplantation refused on this basis.

Research motivation

It is clinically important to determine whether ALF associated with HLH is primarily from intrinsic liver injury vs secondarily from extrahepatic injury from HLH to help determine the potential efficacy of liver transplantation for ALF associated with HLH. ALF from direct liver injury from HLH might be treatable by liver transplantation, whereas liver injury secondary to extrahepatic damage from HLH would be unlikely to be successfully treated by liver transplantation.

Research objectives

To determine whether ALF associated with HLH is due primarily to intrinsic liver injury vs secondary to extrahepatic injury from HLH to help determine whether liver transplantation is potentially warranted. This study involves systematic review of the literature on liver injury associated with HLH. Additionally, a case report is presented to illustrate that a patient with ALF secondary to HLH may be an inappropriate candidate for liver transplantation, despite a high model for end stage liver disease (MELD) score, when the patient has other likely lethal complications of HLH, such as overwhelming sepsis, septic shock, or disseminated intravascular coagulation secondary to explosive immune activation from HLH.

Research methods

Literature on hepatic manifestations of HLH, including liver injury or ALF secondary to HLH, was systematically reviewed by computerized search using PubMed for articles with the following medical subject headings/keywords ("hemophagocytic lymphohistiocytosis" or "HLH") AND ("liver" or hepatic), and by reviewing sections on HLH in standard pathology textbooks/monographs. Articles before 1980 were selectively excluded because clinical evaluations before 1980 often lacked currently required clinical tests of hepatic function, radiologic imaging of hepatic anatomy, and modern criteria for HLH. Large clinical trials, meta-analyses, systematic reviews, and controlled trials were assigned higher priority than review articles or small clinical series, and individual case reports were assigned the lowest priority. Two authors independently performed a literature search, and decided on which articles to incorporate into this review according to article priority based on consensus. Dr. Cappell has considerable experience in conducting systematic reviews, with 4 systematic reviews in peer-reviewed journals, indexed in PubMed, published during the last 2 years, and with a PhD in neurophysiology that involved 5 years of training and research in biomedical statistics. A case report is presented to illustrate that a patient with ALF secondary to HLH may be an inappropriate candidate for liver transplantation, despite a high MELD score, when the patient has other, likely lethal, complications of HLH. This case report was thoroughly analyzed based on the medical chart, including re-review of original endoscopic photographs by an expert endoscopist, radiologic images by an expert radiologist, and pathologic slides by an expert pathologist.

Research results

This systematic review shows that HLH frequently involves the liver. It frequently causes aspartate aminotransferase levels > 2 times the upper limit of normal, frequently causes LDH levels > 2 times the upper limit of normal,

and very frequently causes profound direct hyperbilirubinemia. Likewise, patients commonly present with moderately severe hypertriglyceridemia from cholestasis, and hypoalbuminemia from decreased liver synthetic function. The liver injury is associated with overproduction of cytokines, severe hepatic inflammation, and potentially hepatic necrosis. When patients present with imminent or evident ALF predominantly from direct liver involvement from HLH, liver transplant may be potentially indicated. In such cases, the ALF may be documented by a MELD score > 24-26, and the ALF should contribute significantly to the poor prognosis. However, the currently reported case illustrates that patients may be poor candidates for liver transplantation from ALF secondary to HLH, when the HLH is likely irreversible, or if the patient has potentially lethal, major, comorbidities (systemic complications) from the HLH. For example, the currently reported patient had highly lethal, comorbidities of overwhelming sepsis, shock, and disseminated intravascular coagulopathy, that precluded liver transplantation despite having a MELD score of 33, which would normally have qualified the patient for liver transplantation. This work illustrates that decisions on liver transplantation in patients with HLH depend upon the presence of imminent or evident ALF, the potential reversibility of the HLH, and the absence of severe and most likely fatal comorbidities associated with HLH.

Research conclusions

HLH frequently involves the liver and can directly cause severe liver injury or ALF from hepatic inflammation and hepatic necrosis from explosive immune hyperactivation related to hypercytokinemia. HLH can also indirectly cause liver injury from extrahepatic disease secondary to HLH, such as overwhelming sepsis, septic shock, hypoxemia, and disseminated intravascular coagulation. It is important to determine the relative contributions of these two alternative mechanisms of liver injury when contemplating liver transplantation for HLH. Intrinsic liver disease may respond to liver transplantation, whereas liver injury secondary to extrahepatic causes, such as septic shock, is unlikely to respond to liver transplantation if the extrahepatic causes are irreversible.

Research perspectives

This work places in perspective that decisions on liver transplantation for ALF associated with HLH must consider not only the functional status of the liver, as indicated by the MELD score, but the etiology of the liver injury. When the liver injury is directly from the HLH (e.g., hepatic inflammation and necrosis from hypercytokinemia) liver transplantation may be considered, whereas when the liver injury is secondary to extrahepatic (other organ) failure (e.g., overwhelming sepsis, hyperimmune activation in other organs, disseminated intravascular coagulation, or respiratory failure) liver transplant is unlikely to be successful. This concept is illustrated by a case report wherein the ALF associated with HLH was secondary to extrahepatic causes and not amenable to liver transplantation, and by systematic review of liver injury from HLH. This work may prove useful to clinicians in decisions on whether to perform liver transplantation for ALF associated with HLH.

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Protecting kidneys in liver transplant patients: A pathway to preventive interventions

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Abstract

Acute kidney injury (AKI) is a frequent postoperative complication after liver transplantation. The etiology is multifactorial, including perioperative renal status, surgery related events, and postoperative immunosuppression therapy. The role of renal hypoperfusion and hepatic ischemia-reperfusion injury as causes of early AKI are now being increasingly recognized. Further studies should focus on therapies that would attenuate this injury.

Key words: Acute kidney injury; Liver transplantation; Hepatic ischemia-reperfusion injury; Marginal grafts; Small interfering ribonucleic acid

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Core tip: Acute kidney injury early post liver transplantation is a major cause of morbidity and mortality. The etiology is multifactorial. The renal hypoperfusion and hepatic ischemia-reperfusion injury are being increasingly recognized. Further studies need to focus on therapies that would attenuate this injury.

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TO THE EDITOR

Acute kidney injury (AKI) is a frequent and serious

postoperative complication in the early period after liver transplantation (LT) and is believed to be a major cause of morbidity and mortality. The incidence of postoperative AKI varies widely, from 17% to 95% of the recipients, with some requiring renal replacement therapy. The etiology is suggested to be multifactorial and related to almost all aspects of perioperative management. The factors that have been widely discussed in the literature concentrate on the recipient factors including their preoperative renal status, surgery-related events, including blood loss, hypotension, and postoperative immunosuppression therapy effects, such as calcineurin inhibitor-induced vasoconstriction. Based on these independent risk factors, many preventive interventions to reduce the risk of renal injury have been instituted.

Renal hypoperfusion during liver transplantation has been recognized as an important cause of AKI for a long time and studies have estimated, that ischemic and hypovolemic acute tubular necrosis are the cause for AKI in more than a third of liver transplant recipients^[1]. Recent progress has been made in the field of open heart surgery, another surgical procedure, with a high incidence of ischemic and hypovolemic AKI. A small interfering ribonucleic acid (siRNA) targeting p53 developed by Quark Pharmaceuticals, has been developed for prevention of Delayed Graft Function following renal transplantation but we now have evolving evidence that use of this siRNA during cardiac surgery results in a 26% relative risk reduction in the incidence of AKI^[2]. Consideration should be given to also study the use of this siRNA in liver transplantation.

Presently, the role of hepatic ischemia-reperfusion injury (IRI) in the pathogenesis of AKI early after liver transplantation is being increasingly recognized. IRI is defined as cellular damage after the reperfusion of the previously viable ischemic tissues, *i.e.*, liver allograft. Reperfusion of the liver allograft is associated with increased reactive oxygen species production and inflammation, resulting in renal tubular cell injury and death. The severity of the IRI and the postoperative systemic inflammatory response, which is the common pathway in organ dysfunction, including kidney injury, has been linked to the use of the marginal organs, including liver grafts from older donors, liver grafts with prolonged cold ischemia time, graft steatosis, split liver allografts, and donation after circulatory death (DCD). Leithead *et al.*^[3] demonstrated that in the immediate postoperative period DCD liver transplantation was

associated with an increased incidence of AKI [DCD 53.4%; donor after brain death (DBD) 31.8%, $P = 0.004$]. In their study increased peak perioperative aspartate aminotransferase (AST), a surrogate marker of hepatic ischemia-reperfusion injury, was the only consistent predictor of renal dysfunction after DCD transplantation (OR 7.44, 95%CI: 2.78–19.88, $P < 0.001$). Rahman *et al.*^[4] showed that peak serum AST was the only consistent predictor of AKI in multivariate analysis (OR 1.001, 95%CI: 1.00–1.001, $P = 0.02$), suggesting that hepatic ischemia-reperfusion injury may play a critical role in the pathogenesis of post-transplant renal dysfunction. In various studies, AKI and IRI were associated with a longer time to extubation, increased length of intensive care unit stay and reduced patient and graft survival. AKI has also been shown to be a risk factor for chronic kidney disease^[3,4].

Recently, the growing demand for organs, has necessitated the increased use of more marginal liver grafts, potentially leading to the increasing incidence of the IRI and AKI. Given the high incidence of post-LT AKI and its significant impact on recipient survival and complications, further research should be aimed at the attenuating hepatic ischemia-reperfusion injury, while investigating its effects on preventing post-LT AKI and eventually improving outcomes. Because preventive strategies are limited and the evidence is still lacking, further studies should focus on therapies, such as liver machine perfusion and pharmacologic therapies similar to siRNA approaches, that silence genes associated with ischemic AKI or ischemia-reperfusion injury, and participation in clinical trials would elucidate the translational research from the bench to the bedside.

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