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HEPATITIS B (JK LIM, SECTION EDITOR)

Role of HBsAg Testing in the Management of Patients with Chronic HBV

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Abstract

Purpose of Review HBsAg has been an important serological marker for the diagnosis of hepatitis B virus (HBV) infection. Recently, HBsAg quantification has been utilized to predict the disease activity and the response to antiviral treatment. This review aims to update the clinical utility of HBsAg quantification in the management of chronic hepatitis B (CHB).

Recent Findings HBsAg level varies across different phases of the natural history of CHB, highest in the immune-tolerant phase and lowest in inactive carriers. HBsAg level is useful to stratify the risk of hepatocellular carcinoma in low-viremic patients. HBsAg level also helps predict the HBsAg loss in HBeAg-negative patients. Moreover, baseline level and on-treatment kinetics of HBsAg are associated with the treatment response to pegylated interferon and nucleos(t)ide analogs in both HBeAg-positive and HBeAg-negative patients. Finally, HBsAg level can serve as a therapeutic endpoint marker for evaluating novel curative agents against HBV infection.

Summary HBsAg quantification can help guide the management of CHB.

Keywords Hepatitis B virus · Chronic hepatitis B · HBsAg quantification · Antiviral therapy · Functional cure

Introduction

The discovery of hepatitis B surface antigen (HBsAg), initially called Australia antigen, by Dr. Blumberg and his colleagues in 1967, soon resulted in the identification of the causative agent of serum hepatitis, later formally named hepatitis B, and inaugurated the golden age of research on the molecular biology of hepatitis B virus (HBV) [1]. This groundbreaking finding eventually led to the development of HBV vaccines and their subsequent nationwide implementation by many endemic countries including Taiwan, a critical procedure for the global control and elimination of HBV [2]. Persistence of hepatitis B surface antigenemia for more than 6 months indicates the chronicity of HBV infection, whereas HBsAg seroclearance or seroconversion, defined as loss of HBsAg with or without the gain of anti-HBs, suggests the resolution of HBV infection. Nevertheless, a small proportion of people with resolved HBV infection still have residual HBV DNA in the serum or liver, known as occult HBV infection (OBI) [3•].

Chronic hepatitis B (CHB) exhibits a unique natural history with distinctive chronological phases, particularly in individuals infected via vertical or perinatal transmission, reflecting the complex interactions between HBV and the host immunity [4, 5]. The level of HBsAg varies widely among these clinical phases, but usually declines along with time. Of note, although most HBV patients carry HBsAg throughout the entire lifetime, a certain portion of them may experience spontaneous HBsAg loss at later decades of life. It is estimated that around 40% of CHB patients have persistent or intermittent hepatitis flares and eventually suffer from severe clinical consequences, such as advanced fibrosis/cirrhosis, hepatocellular

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carcinoma (HCC), or hepatic decompensation [6]. Prevention of the adverse clinical outcomes of CHB patients requires timely identification and antiviral treatment for those in need. Recently, the level of serum HBsAg has been proposed as a good biomarker, in particular combined with HBV DNA levels, to help distinguish disease states of CHB [7••]. The introduction of sensitive and accurate assays of HBsAg quantification has lent great momentum for its rapidly growing utility in the management of CHB. Several recent studies have clarified the role of serum HBsAg levels in predicting clinical outcomes during the natural history and antiviral treatment. Moreover, recent enthusiasm in cure of HBV also emphasizes the critical role of HBsAg seroclearance [8••]. In this concise review, we will summarize and discuss the utility of HBsAg quantification in the management of CHB.

A Brief Overview of HBsAg

The source of HBsAg production in infected hepatocytes is affected by the HBeAg status. Virologically, serum HBsAg results not only from the transcription of covalently closed circular DNA (cccDNA), but also from the integrated HBV genomes. The former is the replicative template for production of infectious HBV, whereas the latter is defective in producing most viral proteins except for HBsAg [7••, 9]. A previous study in chimpanzees discovered that the events of HBV integration were uncommon in HBeAg-positive phase of chimpanzees with chronic HBV infection, but they accumulated along with time in HBeAg-negative phases. As a result, the majority of HBsAg was produced from cccDNA in HBeAg-positive chimpanzees, whereas the integrated HBV genomes were the major source of HBsAg production in HBeAg-negative chimpanzees. However, in humans, the HBV integration events are not uncommon [10]. Consistent with this observation, prior studies in CHB patients showed that HBsAg level correlated well with levels of serum HBV DNA and intrahepatic cccDNA only in HBeAg-positive patients, but the correlation between HBsAg level and levels of serum HBV DNA and intrahepatic cccDNA was poor in HBeAg-negative patients [11, 12].

Role of HBsAg in the Natural History of CHB and Prediction of Clinical Outcomes

HBsAg Levels in the Natural History of CHB

The natural history of CHB can be divided into five distinctive chronological phases, immune-tolerant, immune-clearance, residual inactive carrier, reactivation of HBeAg-negative hepatitis, and HBsAg seroclearance/seroconversion phases. The transition from immune-tolerant phase to immune-clearance phase is probably triggered by the awakening of seemingly dormant host immunity against HBV, and the majority of HBsAg carriers subsequently undergo HBeAg seroconversion and turn into an inactive carrier state [5, 13]. HBeAg seroconversion is one of the landmarks in the natural history of CHB, often confers a favorable clinical outcome. Among HBeAg seroconverters, some will eventually achieve HBsAg seroclearance or seroconversion. Previous longitudinal studies have estimated the annual rate of HBsAg loss, ranging from 0.5 to 2.3% [14–19]. A recent meta-analysis reported that HBsA loss is around 1.02% annually [20••]. Patients who lose HBsAg with the absence of advanced fibrosis at the time of HBsAg loss have the most favorable outcomes.

It has been shown that HBsAg levels vary across the natural history of HBV infection [21–24]. The dynamic change of HBsAg levels along with other virological markers in different phases of the natural history of CHB is summarized in Fig. 1. HBsAg level was higher in HBeAg-positive patients than in HBeAg-negative patients. Among HBeAg-positive patients, patients in the immune-tolerant phase have the highest HBsAg level, approximately 5 log IU/mL. HBeAg-positive patients with active disease and those undergoing HBeAg seroconversion had comparable levels of HBsAg, approximately 4 log IU/mL. Among HBeAg-negative patients, patients with active disease exhibited higher HBsAg level than inactive carriers. In addition, combined single-point quantification of HBsAg (< 1000 IU/mL) and HBV DNA (≦ 2000 IU/mL) can accurately identify inactive carriers [25–27]. The combined criteria identified the inactive carriers with 94.3% diagnostic accuracy and 87.9 positive predictive value (PPV) in genotype D. The same criteria were also applied for identifying inactive carriers with 78% diagnostic accuracy and 83% PPV in genotype B and C-infected carriers of the REVEAL cohort [25, 27].

HBsAg Level in Predicting Clinical Outcomes

HBeAg-Positive Patients

HBsAg is produced not only from cccDNA, but also from integrated HBV. However, the proportion of either source from which HBsAg production results is influenced by the phases of HBV infection, particularly by the HBeAg state. Previous studies have demonstrated that, although serum HBsAg level was correlated well with levels of serum HBeAg, serum HBV DNA, and intrahepatic cccDNA in HBeAg-positive patients, their correlation is poor in HBeAg-negative individuals [11, 12, 28]. As mentioned above, HBsAg level declines from the highest in the immune-tolerant phase to the lowest in the inactive carriers, suggesting an immune control over HBV infection. Meanwhile, the HBsAg levels of HBeAg-positive and HBeAg-negative immune-active patients are in-between. Nevertheless, baseline or kinetics of HBsAg levels fail to predict spontaneous HBeAg seroconversion [21].

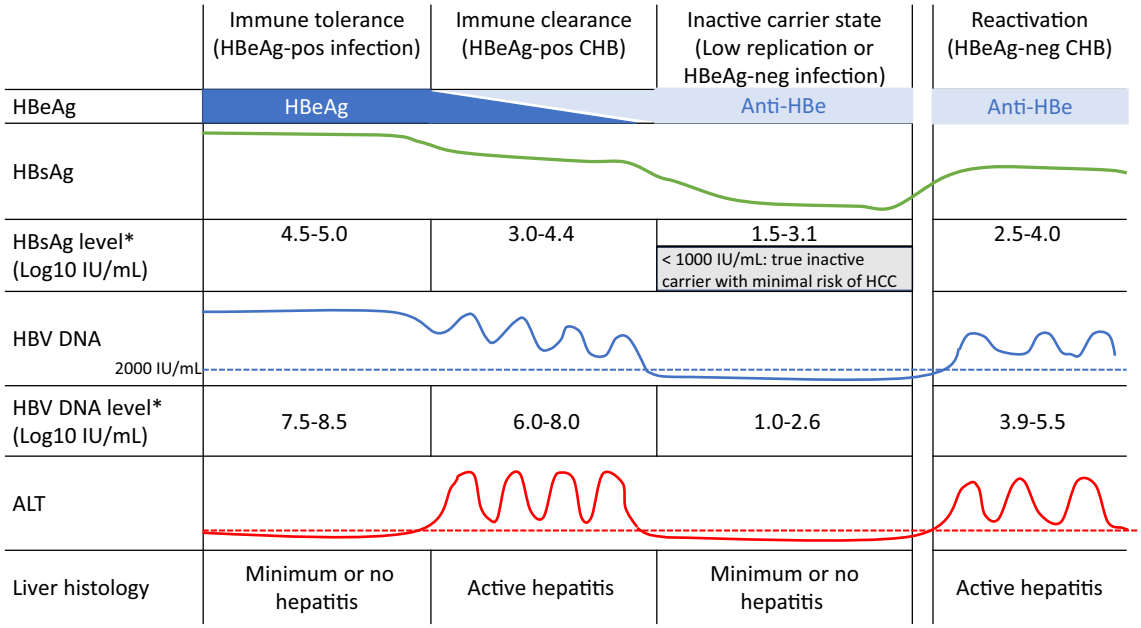

Fig. 1 The dynamic change of HBsAg levels with other virological markers in different phases of CHB

The longitudinal study of 390 Taiwanese spontaneous HBeAg seroconverters found that serum HBsAg level at 1 year after HBeAg seroconversion could predict the HBsAg loss within 6 years. Compared with patients with HBsAg levels ≧ 1000 IU/mL, the hazard ratios of HBsAg loss for patients with HBsAg of 100 to 999 and < 100 IU/mL were 4.4 and 24.3, respectively. In patients with low HBV DNA (< 200 IU/mL), an HBsAg level < 100 IU/mL predicted HBsAg loss within 6 years with a diagnostic accuracy of 91.5% and PPV of 45.5% [19]. Additionally, HBsAg level also helped to predict the risk of HBeAg-negative hepatitis in spontaneous HBeAg seroconverters with low viremia. The hazard ratio of HBeAg-negative hepatitis for patients with higher HBsAg level ≧ 1000 IU/mL vs. HBsAg level < 1000 IU/mL was 4.1 among those with HBV DNA < 2000 IU/mL [29].

HBeAg-Negative Patients

The REVEAL-HBV study clearly demonstrated that the serum HBV DNA level was strongly associated with the risk of HCC over time [30]. In HBeAg-negative patients, serum HBsAg level could complement serum HBV DNA level and help predict long-term clinical outcomes of CHB, including HBsAg seroclearance, the progression of liver diseases, and the risk of HCC, particularly in those with low viremia (HBV DNA < 2000 IU/mL) (Fig. 2a). HBeAg-negative patients with high HBsAg level (> 1000 IU/mL) had a higher risk of HCC. In a hospital-based Taiwanese cohort (ERADICATE-B) of HBsAg carriers, the hazard ratios for HBeAg-negative hepatitis and HCC in patients with levels of HBsAg ≧ 1000 IU/mL versus < 1000 IU/mL were 1.5 and 13.7, respectively, among HBeAg-negative patients with low viral load (HBV DNA < 2000 IU/mL) [31, 32]. The hazard ratios for HBsAg loss in low-viremic patients with HBsAg levels of 100–999, 10–99, and < 10 IU/mL versus > 1000 IU/mL were 2.5, 2.8, and 13.2, respectively [33] (Fig. 2b). Consistently, the study on the community-based REVEAL-HBV cohort also reported that the baseline inactive carrier status (HBV DNA < 2000 IU/mL and HBsAg < 1000 IU/mL) predicted a low risk of liver cirrhosis and HCC and a high chance of HBsAg seroclearance with the adjusted hazard ratios of 0.36, 0.36, and 6.97, respectively [27]. Actually, a very low serum HBsAg level (< 100 IU/mL) could identify the HBV patients with a minimal risk of HCC and a high probability of spontaneous HBsAg seroclearance [26, 27, 32, 34, 35].

Utility of HBsAg Levels in Antiviral Treatment

Pegylated interferon (IFN) and nucleos(t)ide analogs (NAs) are the recommended agents for CHB treatment. Pegylated IFN controls HBV through both the direct viral suppression and indirect host immune response while the NA treatment targets the viral polymerase and is designed to suppress the viral replication. A finite 12-month course of pegylated IFN can induce long-term therapeutic response in a small portion of patients. In contrast, NA therapy usually requires long-term or even lifelong medication for viral suppression in most patients. Due to their fundamentally different mechanisms in suppressing HBV, quantitative HBsAg levels before, during, and at the end of treatment (EOT) may have different predictive roles in treatment response to pegylated IFN or NAs as summarized in Table 1 according to current available evidence and will be introduced later in the article. HBsAg seroclearance is widely accepted as a surrogate marker for

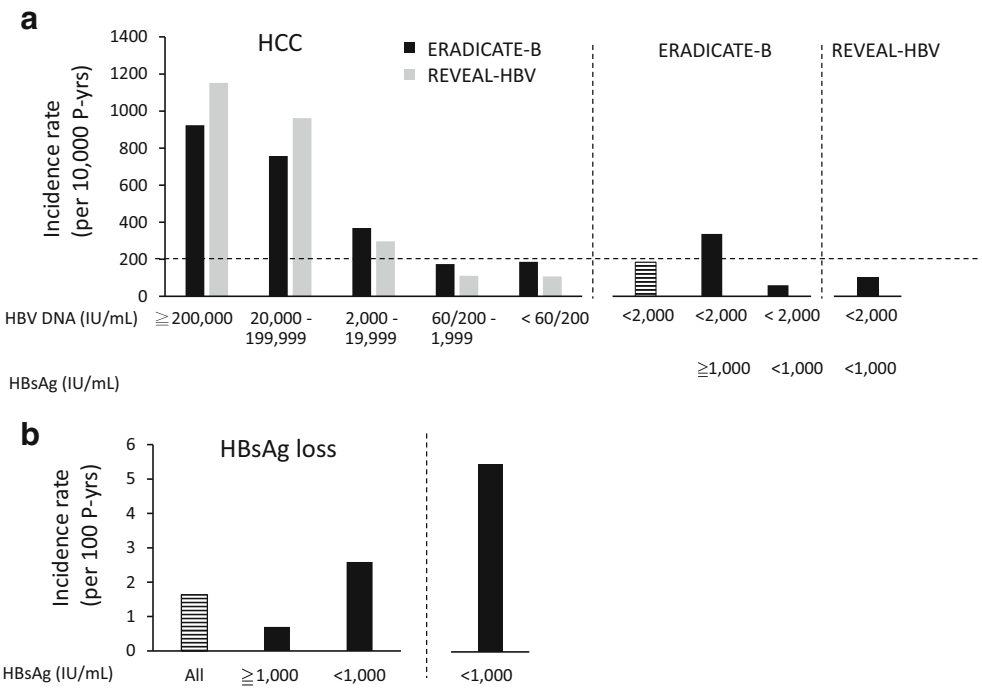

Fig. 2 The predictive role of HBsAg level in the risk of HCC and the chance of HBsAg loss. **a** The incidence of HCC across different levels of HBV DNA and HBsAg (ref. 27, 30, and 31). **b** The incidence of HBsAg seroclearance in HBeAg-negative patients with HBsAg > 1000 IU/mL and those with HBsAg < 1000 IU/mL (ref 19 and 27)

functional cure in HBV patients undergoing a successful treatment, but it is a rare event with an estimated annual rate of 1.02% in the natural course of disease [20••]. The recent meta-analysis suggests no significant association between HBsAg seroclearance rate and prior treatment. However, patients treated with pegylated IFN had a significantly higher rate of HBsAg seroclearance (1.80%) compared to those receiving NA treatment (0.80% for entecavir and 0.65% for lamivudine-treated patients). These conflicting results may be due to the exclusion of multiple important pegylated IFN studies with the patient number below 200, so it may not draw definite conclusions until more large studies addressing this issue [45].

The Role of HBsAg Levels in Pegylated IFN Treatment

HBeAg-Positive Patients

The HBsAg quantification is valuable in prediction of treatment success of pegylated IFN as well as its early discontinuation in patients with low response rate because the risk of adverse effects outweigh the therapeutic benefits. The predictive value of on-treatment HBsAg levels for HBeAg seroconversion is modest in HBeAg-positive CHB patients receiving pegylated IFN treatment. The PPV of HBsAg ≦ 1500 IU/mL at weeks 12 and 24 on-treatment were 57% and 54%, respectively, for HBeAg seroconversion at 6-month post-treatment in the retrospective analysis of a large randomized study [46]. These "early responders" who will benefit from pegylated IFN treatment were also supported by a later randomized trial, which defined them by HBsAg < 1500 IU/mL or HBV DNA $< 10^5$ copies/mL at week 24 [36]. High rates (17.6% and 20.3%) of HBsAg clearance at 6-month post-treatment were also achieved when applying the same criteria at week 12 and 24. The highest positive prediction rate among trials for a sustained virological response (defined by seroconversion of HBeAg and HBV DNA ≦ 10,000 copies/mL at the end of treatment course and sustained after 12-month post-treatment) was 75% in a relatively smaller scale cohort using HBsAg ≦ 300 IU/mL at 6 months of treatment combined with a reduction of HBsAg greater than 1 log at the same time [37]. The scale of on-treatment HBsAg decline was also studied in a randomized trial showing responders (HBeAg loss and HBV DNA < 10,000 copies/mL at 26-week post-treatment) had more significant decline in HBsAg than non-responders (3.3 versus 0.7 log IU/mL at week 52) [47].

On the other hand, the on-treatment HBsAg level is excellent in predicting treatment failure. Hence, the term "stopping rule" was used for discontinuation of pegylated IFN treatment. In a meta-analysis, for those with HBsAg levels above 20,000 IU/mL (genotype B, C) or no decline (genotype A, D) at week 12, the negative predictive values (NPV) were 97–100% and 92–98%, respectively. For all the genotypes, the cutoff point of HBsAg > 20,000 IU/mL at week 24 achieved an NPV of 99–100% for treatment response defined by HBeAg loss with HBV DNA < 2000 IU/mL at 6-month post-treatment [38].

HBeAg-Negative Patients

For the prediction of treatment response of HBeAg-negative CHB patients, those who had an HBsAg decline

Table 1 The predictive role of quantitative HBsAg in the treatment response and clinical outcomes of antiviral therapy

	Utility of HBsAg level	Treatment response/clinical outcomes	Ref
Pegylated IFN			
HBeAg-positive	HBsAg ≦ 1500 IU/mL at week 12	57% PPV for HBeAg seroconversion at 6 months post-treatment, and 17.6% HBsAg loss	[36]
	HBsAg < 300 IU/mL and HBsAg decrease > 1 log at 6-month on-treatment	sustained virological response (HBeAg seroconversion and HBV DNA ≦ 10,000 copies/mL at EOT and at 12 months post-treatment)	[37]
	HBsAg no decline at week 12	92–98% NPV for treatment response (HBeAg loss with HBV DNA < 2000 IU/mL at 24 weeks post-treatment) (genotype A and D)	[38]
	HBsAg > 20,000 IU/mL at week 12	97–100% NPV for treatment response (genotypes B and C)	[38]
	HBsAg > 20,000 IU/mL at week 24	99–100% NPV for treatment response (all genotypes)	[38]
HBeAg-negative			
	HBsAg > 10% decline from baseline at 12 weeks and 24 weeks	47.2% and 43.4% PPV for HBV DNA ≦ 2,000 IU/mL at 1-year post-treatment; 22.6% and 22.4% PPV for HBsAg clearance at year 5 post-treatment	[39]
	HBsAg level < 10 IU/mL at EOT and on-treatment decline > 1 log IU/mL	Associated with sustained HBsAg clearance 3 years post-treatment.	[40]
	EOT genotype-specific cutoffs of HBsAg levels (A (< 400 IU/mL), B (< 50 IU/mL), C (< 75 IU/mL), and D (< 1000 IU/mL)	75%, 47%, 71%, and 75% Long-term virological response (HBV DNA ≦ 2000 IU/mL) at 5-year post-treatment), respectively	[41]
	No HBsAg decline and < 2 log IU/mL HBV DNA decline at week 12	100% NPV for sustained response defined byHBV DNA level < 10,000 copies/mL and normal ALT levels at week 72.	[42]
NA			
HBeAg-positive	HBsAg decrease of 1 log at week 12 or 24	35–45% PPV and 94–97% NPV for HBsAg loss in TDF-treated HBeAg-positive patients	[43]
HBeAg-negative	HBsAg < 100 IU/mL at EOT	Low off-therapy virological relapse (9–19%) and clinical relapse (15–30%) and high off-therapy HBsAg loss rates (21–59%)	[44]

≧ 10% $\log_{10}$ IU/mL from baseline at weeks 12 and 24 resulted in higher sustained response and HBsAg seroclearance up to 5-year post-treatment. The PPVs for HBV DNA ≦ 2000 IU/mL at 1-year post-treatment were 47.2% and 43.4%, respectively. The PPVs for HBsAg clearance at year 5 were 22.6% and 22.4%, respectively [39]. The EOT HBsAg level < 10 IU/mL was reported to have a 52% probability for HBsAg loss 3 years after treatment and HBV DNA suppression to < 400 copies/mL 6 months after treatment [40]. The genotype-specific EOT HBsAg cutoff levels could also achieve good positive prediction for long-term virological response with genotypes A (< 400 IU/mL, PPV 75%), B (< 50 IU/mL, PPV 47%), C (< 75 IU/mL, PPV 71%), and D (< 1000 IU/mL, PPV 75%) [41].

The prediction, however, for the poor response to pegylated IFN also performed well in HBeAg-negative patients. The original PARC trial identified a stopping rule for genotypes A and D with a 100% NPV for sustained response defined by HBV DNA level < 10,000 copies/mL and normal ALT levels at week 72 [42]. The PARC stopping rule for patients with the absence of HBsAg decline and < 2 log IU/mL HBV DNA decline at week 12 was later validated in a study with pooled genotypes achieving 95% NPV and excelled for genotype D with a NPV of 100% [48].

The Role of HBsAg Levels in NA Treatment

Kinetics and Cutoffs of HBsAg during NA Therapy

During NA treatment, the discrepancy between the slow decline of HBsAg and rapid reduction of HBV DNA can be explained by the mechanisms involved in HBsAg production and antiviral treatment [49]. HBsAg is produced from both cccDNA and integrated HBV DNA in infected hepatocytes. However, NA only targets the reverse transcription process of pre-genomic RNA, but not cccDNA or integrated HBV DNA. Hence, NA treatment does not directly affect the HBsAg production and secretion in comparison with that of immune modulation by IFN treatment. For HBeAg-positive disease, prior studies suggested that the majority of HBsAg come from cccDNA and a small portion from integrated viral DNA. On the contrary, for HBeAg-negative disease, a significant portion of HBsAg comes from integrated viral DNA [50]. Under NA treatment, the greater decline rate of HBsAg was noted in HBeAg-positive patients compared to HBeAg-negative patients [51]. The rapid on-treatment decline of HBsAg (≧ 0.5 log10 IU/mL at 24 week and ≧ 1 log10 IU/mL at year 1) serves as a predictor of subsequent HBsAg seroclearance at year 3 in patients treated with telbivudine [52]. An HBsAg decrease of 1 log at week 12 or 24 under tenofovir treatment also revealed the NPV of 94–97% and PPV of 35–45% for

HBsAg loss [43]. Genotype A or D, Caucasian, elevated baseline ALT and AFP were reported to be associated with rapid HBsAg decline and the latter two suggested immune-mediated hepatocytolysis in play [43, 51–53].

For the prediction of HBsAg loss in genotypes B and C, an HBsAg cutoff of < 200 IU/mL at any single time point was proposed for high NPV (> 96%) and when combined with IU/mL with dynamics of 1 log IU/mL or more decrease in HBsAg level over a 2-year period has a PPV for 97–100% [54], and also achieved optimal AUROC of 0.867 when combined with a 0.5 log IU/mL reduction of HBsAg [55].

Stopping NA and End-of-Treatment Relapse Prediction

The cessation of NA treatment gains more attention in recent years due to the low probability of HBsAg loss requiring lifelong medication in most patients, long-term safety profiles not elucidated, cumulative cost, and decreasing adherence. The virological and biochemical relapse is not uncommon in the patients after stopping NA treatment. Several studies discovered the utility of HBsAg levels as a marker to predict the durability of sustained remission and risk of relapse after NA discontinuation. The hazard ratio per log IU/mL increment of HBsAg was 2.47 for clinical relapse (HBV DNA > 2000 IU/mL and ALT > 2× upper limit of normal) and 1.80 for virological relapse (HBV DNA > 2000 IU/mL). Relapse was not found with HBsAg < 10 IU/mL in a prospective study with a relatively small number of patients [56]. The EOT quantitative HBsAg (qHBsAg) was associated with sustained viral response (adjusted OR 0.32 every $\log_{10}$ qHBsAg IU/mL), undetectable HBV DNA levels at 12 months off-therapy [57]. On the other hand, the EOT HBsAg < 100 IU/mL can predict the earlier accelerated HBsAg loss in 12 months in comparison to those ≧ 100 IU/mL after cessation of NA (91.7% vs 58.3%) which also predicts sooner HBsAg loss (5.5 vs 21.9 months) [58••].

A systemic review highlighted the stopping rule and off-therapy monitor plan in HBeAg-negative CHB patients treated with NA. The recommended cessation strategy is NA ≧ 2 years without genotypic resistance, plus consolidation therapy ≧ 12–18 months especially if HBsAg level < 200 IU/mL. The off-therapy follow-ups include frequent tests for ALT and HBV DNA. With rising ALT or > 5× ULN suspecting relapses, a more frequent plan for testing ALT, bilirubin, and PT should be implemented [59]. Another systemic review suggests that a durable virological response is much higher in the virological remission duration > 24 months before stopping NAs [60]. However, a recent systemic review suggests a more stringent EOT NA cessation rule. The proposed optimal levels of HBsAg were < 100 IU/mL with low rates of virological relapse (9–19%) and clinical relapse (15–30%) and high off-therapy HBsAg loss rates (21–59%) [44]. This cutoff was also supported by an earlier prospective study suggesting that only EOT HBsAg level was associated with SVR defined by undetectable HBV DNA levels at 12 months off-therapy in multivariable analysis. After stratification, the cumulative incidence of SVR was significantly higher in off-therapy HBsAg cutoff < 100 IU/mL than 100–1000 or > 1000 IU/mL [57, 61]. Besides a fixed off-treatment HBsAg level, the HBsAg dynamics after the cessation of NA therapy was also associated with relapse. A one log increase of HBsAg level has been associated with adjusted hazard ratio (HR) of 2.10 in virological relapse (HBV DNA > 2000 IU/mL) and HR of 2.32 in clinical relapse (ALT > 80 IU/mL and HBV DNA > 2000 IU/mL) [62]. A recent retrospective study addressing the EOT kinetic issue revealed the gradual decrease of HBsAg followed by a precipitous HBsAg decline (> 0.5 $\log_{10}$ IU/mL in 1 year) prior to HBsAg loss in 92.9% of the patients. At the same time, an EOT qHBsAg < 100 IU/mL predicts earlier (< 12 months) precipitous HBsAg decline and sooner HBsAg loss [58••].

The Role of HBsAg Levels in NA and Pegylated IFN Combination Treatment

The combination or switch therapy of NA and pegylated IFN also attracts wide interest both in the studies of HBV-specific immune response and intriguing clinical response. However, the topics are still under debate due to the controversial results from several literatures and lack of long-term follow-up data [63]. Several meta-analysis addressing this issue revealed that combined therapy using pegylated IFN with lamivudine or adefovir was not superior to pegylated IFN monotherapy for HBsAg seroclearance [64, 65•]. A particular meta-analysis discussed combination methods showed that NA-experienced strategies were more likely to have response (11%) than de novo strategy (8%) which is the simultaneous initiation of NA and pegylated IFN. Among the NA-experienced strategies, "switch-to" has higher likelihood of HBsAg loss than "add-on" [65•]. The aforementioned results were largely based on the "New Switch Study", which also identified baseline HBsAg < 1500 IU/mL and HBsAg < 200 IU/mL at week 24 having highest rates of HBsAg loss with 51.4% by 48 weeks of treatment and 58.7% by 96 weeks of treatment [66]. Similar predictive values of both baseline HBsAg < 1500 IU/mL and HBsAg < 200 IU/mL at week 12 were also proposed in the earlier OSST trial [67]. A recent phase IV randomized clinical study with the short course of NA and pegylated IFN combination study failed to demonstrate superiority over pegylated IFN monotherapy, but identified the association of on-treatment HBsAg levels with post-treatment efficacy [68]. The results and subsequent debates concerning the optimal durations of both medication thus urge for future personalized precision therapy [69, 70].

Co-infections

HCV/HBV

Due to the complexity of dominant virus in HCV/HBV co-infection, the treatment strategies differ accordingly, and reactivation of another virus must be monitored carefully. The low and negative HBsAg levels were found in HCV dominant and neither HCV/HBV replicative serology patterns of co-infection [71•]. Currently, co-treatment with direct acting antivirals (DAA) and NA is recommended for HCV/HBV co-infected patients who fulfill the HBV treatment criteria. However, ALT should be monitored in those not fulfilling the HBV treatment criteria [72••, 73••]. HCV patients with positive HBsAg and overt HBV infection are prone to experience HBV reactivation and are subjected to frequent surveillance if treated only by DAAs [74]. Prophylactic use of NA in HBsAg-positive patients until week 12 post-DAA is recommended by EASL guidelines [72••]. Few data demonstrated the role of quantitative HBsAg in HBV/HCV co-infected patients with treatment of DAA plus NA. However, treatment with pegylated IFN and ribavirin which controlled both HBV and HCV was studied extensively in the pre-DAA era. A low baseline HBsAg level < 20 IU/mL was found to be associated with HBsAg clearance. In patients with baseline undetectable HBV DNA, decrease by 50% of HBsAg from baseline compared with week 12 also reduced the likelihood of HBV reactivation with a PPV of 89.5% [75]. A scoring system considering age > 60 years old, male gender, and HBsAg level ≦ 100 IU/mL was also implemented to predict HBsAg seroclearance in HBV/HCV co-infected patients treated with pegylated IFN and ribavirin achieving an AUROC of 71.8%, sensitivity 65.22%, and specificity of 75.32% [76].

HDV/HBV

HDV/HBV co-infection remains an important threat for advanced liver disease. HBsAg plays an essential role in HDV viral replication cycle, so compared to HBV DNA levels, HBsAg levels were relatively high in this situation. However, IFN-alpha is the only clinically available medication for hepatitis D currently. The baseline virological parameters are not associated with sustained virological response [77]. However, at treatment week 24, combination of HDV RNA decrease by 1 log and the absence of HBsAg decline predicts non-responder with a PPV of 83% [78]. In another research, HBsAg < 1000 IU/mL at on-treatment month 6 could discriminate responders, partial responders from non-responders while at the same time, 0.105 log reduction of HBsAg combined with 1.610 log reduction of HDV-RNA from baseline predicted the clearance of HDV-RNA [79]. A qHBsAg-guided therapy was hence proposed and used in real-world patients [80, 81].

HIV/HBV

In the HIV/HBV co-infection, the host immune conditions, especially the levels of CD4 T cells, determine the seroclearance of HBV. A higher baseline HBsAg level was correlated with a lower CD4 counts. A lower HBsAg was noted in patients who received combination antiretroviral therapy (cART) including NAs against HBV compared to untreated subjects. HBsAg levels of HBV/HIV co-infected patients were higher than those of HBV-mono-infected patients despite their similar levels of HBV DNA [82]. Study also noted a correlation between the decline of HBsAg from baseline to last follow-up and the increase of CD4 cells or higher last follow-up CD4 counts [83]. In HBeAg-positive HBV/HIV co-infected patients treated with tenofovir disoproxil fumarate (TDF) as part of highly active antiretroviral therapy (HAART), decline in HBsAg at month 6 was associated with HBsAg loss [84]. In addition, for HBV/HIV co-infection treated by cART, HBeAg-positive patients with on-treatment HBsAg decline ≧ 1 log IU/mL per year had higher HBsAg loss rates. In HBeAg-negative patients, a pre-treatment HBsAg ≦ 100 IU/mL also predicted HBsAg seroclearance [85].

Role of HBsAg in HBV Cure

By the encouraging results of HCV cure and the continual emergence of novel therapeutic strategies with different modes of action, there has been a rising enthusiasm on pursuing HBV cure [8••, 86]. It has been known that persistent cccDNA under antiviral therapy is the major barrier to eradication of HBV infection [87]. Recently, accumulative evidence has emphasized that HBV cure cannot be achieved without targeting the integrated HBV genomes [88]. However, complete HBV cure, defined by the complete eradication of HBV genomes, including cccDNA and integrated HBV DNA, is quite challenging. Alternatively, functional cure with full suppression of HBsAg and HBV DNA production is a more practical goal [89••]. The natural course of CHB has already demonstrated that a small portion of HBsAg carriers can reach spontaneous HBsAg seroclearance, a proof for its clinical feasibility. Although not common, HBsAg clearance can be also achieved in a minor portion of patients receiving current antiviral therapy. Measurement of presence and levels of the HBsAg are critical to evaluate the antiviral efficacy for novel therapeutic strategies aiming to cure CHB. An important question is what the critical thresholds of HBsAg titer decline will be for achieving functional cure by novel curative antiviral therapies. Previous studies on the role of HBsAg levels in predicting the response to pegylated IFN and NAs have shown that more rapid decline of HBsAg and lower on-treatment and EOT HBsAg levels were associated with higher chance of off-therapy sustainability and HBsAg

loss. A recent systemic review concludes that HBsAg <100 IU/mL is associated with lower risk of off-therapy virological relapse [44]. However, it is expected that more stringent criteria of treatment-induced HBsAg decline is required to achieve functional cure. Of note, it is also likely that different curative strategies may apply different HBsAg thresholds that can accurately predict functional cure.

Conclusions

A large body of evidence has clearly demonstrated the utility of HBsAg quantification in the prediction of clinical outcomes of the natural history and antiviral treatment for CHB. In CHB patients with low viral load (< 2000 IU/mL), serum HBsAg level helps to further stratify the risk of progression of liver disease, including cirrhosis and HCC, and identify the inactive carriers with minimal risk. HBsAg quantification also helps to predict the treatment response and off-therapy sustainability of antiviral treatment. Emerging novel therapies aiming for functional cure of HBV should be monitored by measurement of HBsAg levels. In addition to HBsAg loss, the decline of HBsAg or below a certain cutoff value should also serve a surrogate marker to evaluate the efficacy of antiviral therapy.

Compliance with Ethical Standards

Conflict of Interest Tzu-Chan Hong, Hung-Chih Yang and Jia-Horng Kao each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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