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REVIEW

Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: new trick of old dog

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Abstract Using commercial quantitative assays, quantitative hepatitis B surface antigen (qHBsAg) has improved our understanding and management of chronic hepatitis B (CHB). The HBsAg level is highest in the immune tolerance phase, starts to decline during the immune clearance phase, and decreases slowly but progressively after hepatitis B e antigen (HBeAg) seroconversion. The HBsAg level is lowest in individuals with an inactive carrier state but higher in those who develop HBeAg-negative hepatitis. It has been shown that a reduction of HBsAg by 1 log IU/mL or more reflects improved host immune control of HBV infection. A combination of HBsAg <1000 IU/mL and HBV-DNA <2000 IU/mL can identify a 3-year inactive state in a genotype D HBeAg-negative carrier population. In the Asian-Pacific region, where HBV genotypes B and C are dominant, HBsAg levels of ≤10–100 IU/mL predict HBsAg loss over time. As to the prediction of disease progression, low-viremic carriers with HBsAg >1000 IU/mL have been shown to be at higher risks of HBeAg-negative hepatitis, cirrhosis, and hepatocellular carcinoma than those with HBsAg <1000 IU/mL. Although qHBsAg has been widely used in CHB patients receiving pegylated interferon therapy, the HBsAg decline is slow and does not correlate with HBV-DNA levels during nucleos(t)ide analogue (NUC) therapy. However, a rapid HBsAg decline during NUC therapy may identify patients who will finally clear HBsAg. A 6- to 12-monthly assessment of HBsAg level could be considered during NUC therapy. Taking these lines of evidence together, qHBsAg can complement HBV-DNA levels to optimize the management of CHB patients in our daily clinical practice.

Keywords qHBsAg · HCC · NUC · HBV

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Introduction

Although safe and effective vaccines have been available for nearly 3 decades, hepatitis B virus (HBV) infection is still an important public health problem. The clinical manifestations of HBV infection range from acute or fulminant hepatitis to various forms of chronic infection, including an inactive carrier state, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Hepatitis B surface antigen (HBsAg) is the hallmark of HBV

infection and was first discovered by Blumberg and his colleagues, reported in 1968 [2]. Since then, HBsAg has been used as a marker for the diagnosis of HBV infection. Being a qualitative test, HBsAg has been used by most practicing physicians as one of the serological tests to determine the etiology of hepatitis but not to monitor disease progression in patients with chronic HBV infection. In clinical practice, physicians used to rely only on serological qualitative or semi-quantitative markers such as HBsAg and hepatitis B e antigen (HBeAg), as well as liver biochemical tests, to determine the disease phases of HBV carriers; however, since 2007, the quantification of serum HBV DNA level has become a useful marker to predict long-term outcomes of such patients [3]. The recent introduction of HBsAg quantification has attracted much attention for its value in being able to stratify the risk of disease progression and predict treatment response to antiviral therapy in patients with chronic HBV infection. In this review article, recent updates on reports of HBsAg level as a new biomarker to optimize the management of chronic hepatitis B will be discussed.

Life cycle of HBV and synthesis of HBsAg

Having only 3200 base pairs in its genome, HBV is the smallest known DNA virus. Figure 1 illustrates the pathway of HBsAg production in the life cycle of HBV. The replication template of HBV is covalently closed circular DNA (cccDNA), which exists in liver and encodes four overlapping open reading frames (ORFs): S for the surface gene, C for the core gene, P for the polymerase gene, and X for the X gene [4]. The S and C genes also have up-stream regions designated pre-S and pre-C. The whole virion, or Dane particle, is a 42 nm sphere that contains the nucleocapsid and relaxed circular HBV DNA. HBV DNA is synthesized via the reverse transcription of pregenomic RNA, which is also derived from cccDNA. Therefore, cccDNA is the template for both HBV DNA and HBsAg synthesis, while both products are derived from different ORFs of cccDNA (Fig. 1). HBsAg is a glycosylated envelope protein of HBV virions. There are 3 HBsAg proteins—small (S), medium (M), and large (L), and they are translated from pre-S1 mRNA and pre-S2/S mRNA, which are transcribed from S gene of cccDNA. In addition to the HBsAg on the mature virions, there are large numbers of 2 types of non-infectious particles in the sera of HBV carriers: spherical particles and filamentous forms (Fig. 1). Both types of non-infectious subviral particles are composed of HBsAg. The subviral particles do not contain the HBV genome but are secreted at levels far in excess (100–100000 folds) of those of mature virions. Meanwhile, HBsAg can also be synthesized from viral sequences that are integrated randomly into the host genome. Virologically, quantification of serum HBV DNA merely reflects viral replication activity; however, serum HBsAg is produced not only from translated messenger RNAs of transcriptionally active cccDNA but also from integrated HBV DNA sequences. Thus, compared with HBV DNA level, HBsAg level provides different but complementary information that may help us understand patients' infection status more comprehensively.

As to other viral proteins, hepatitis B core antigen (HBcAg) is the nucleocapsid that encloses the viral DNA. When peptides derived from HBcAg and possibly others, such as surface and polymerase proteins, are processed and expressed on the surfaces of liver cells, apoptosis or necrosis of HBV-infected hepatocytes may develop and the virus could be cleared [5].

HBeAg is a circulating peptide derived from the core gene, then modified and secreted from liver cells. It usually

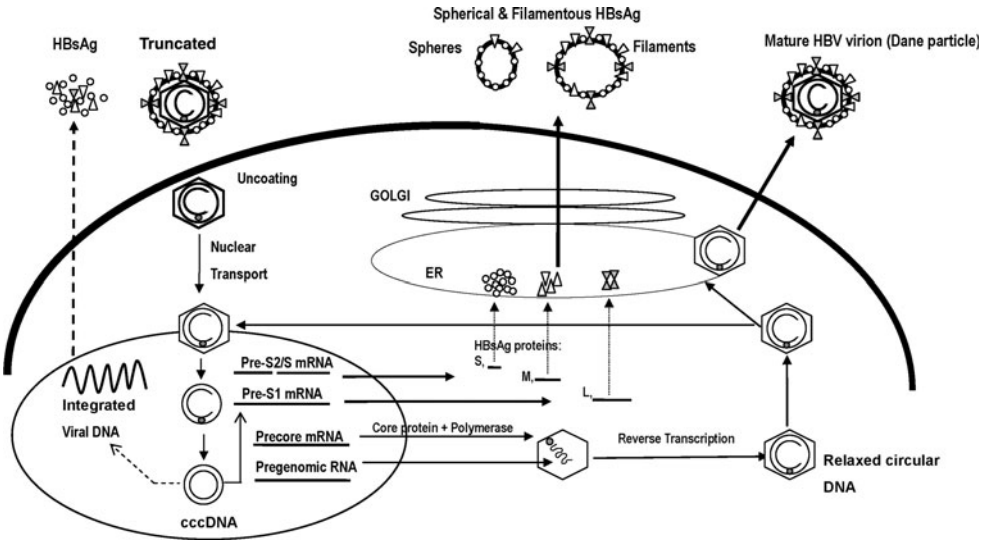

Fig. 1 Pathway of hepatitis B surface antigen (*HBsAg*) production in the life cycle of hepatitis B virus (*HBV*) replication. *cccDNA* covalently closed circular DNA, *ER* endoplasmic reticulum, *PreS2/S mRNA*, *Pre-S1 mRNA*. Adapted from J Hepatol. 2011;55:1121–31

serves as a marker of active viral replication. The long P gene encodes the DNA polymerase; however, because viral replication requires RNA intermediates, the polymerase also provides HBV's reverse transcription function. The X gene encodes the protein that has transactivation activities on the HBV enhancer in aiding viral replication. The X protein can also transactivate other cellular genes that may play a part in hepatocarcinogenesis [5].

Natural history of chronic hepatitis B

In Asia, where HBV infection is hyperendemic, HBV carriers usually acquire the virus perinatally or in early childhood by the age of 2 years; therefore, the age of a given patient can be considered as the duration of infection. On the basis of interactions between virus and host, the natural course of Asian patients with chronic HBV infection can be divided into four chronological phases [6, 7] (Fig. 2). The first is the "immune tolerance phase", which is characterized by active replication of HBV, positivity for HBeAg, and normal-to-low alanine aminotransferase (ALT) levels. The second is the "immune clearance phase", where HBeAg-positive patients have raised ALT levels and a decline of HBV DNA level. In the third "low replication or residual phase", patients lose HBeAg and gain antibody to HBeAg (anti-HBe) with remission of liver disease and are designated as being in an "inactive carrier state". HBeAg seroconversion (losing HBeAg and gaining anti-HBe) is a critical landmark event in the natural history of chronic HBV infection because it usually confers a favorable clinical outcome [8]. However, about 20–30 % of inactive carriers may enter a "reactivation or HBeAg-negative hepatitis phase" during follow-up, which is now recognized as a variant of the immune clearance phase [9–11]. Previous longitudinal studies have indicated that HBeAg-negative hepatitis is a major risk factor for subsequent cirrhosis and HCC development [9, 12–14]. Therefore, early identification of at-risk patients and prompt antiviral treatment are mandatory to prevent or reduce disease progression. In the meantime, there also exist chances for HBV carriers to have HBsAg seroclearance or seroconversion, which is closest to the cure for HBV infection [15].

HBsAg and HBV DNA levels in different phases of chronic hepatitis B

Several cross-sectional studies have compared HBV DNA and HBsAg levels among different phases of chronic hepatitis B (Fig. 2) [16–18]. Although the study populations and HBV genotypes are different, the study results are comparable. Both HBsAg and HBV DNA levels vary at different phases of HBV infection but gradually decrease as HBV carriers become older. The levels are highest in the initial immune tolerance phase when serum ALT level is normal with no or minimal hepatitis activity. The levels become lower during the immune clearance phase and persistently decrease in those who maintain normal ALT levels after HBeAg seroconversion. All the above reports have indicated that the lowest levels of HBsAg and HBV

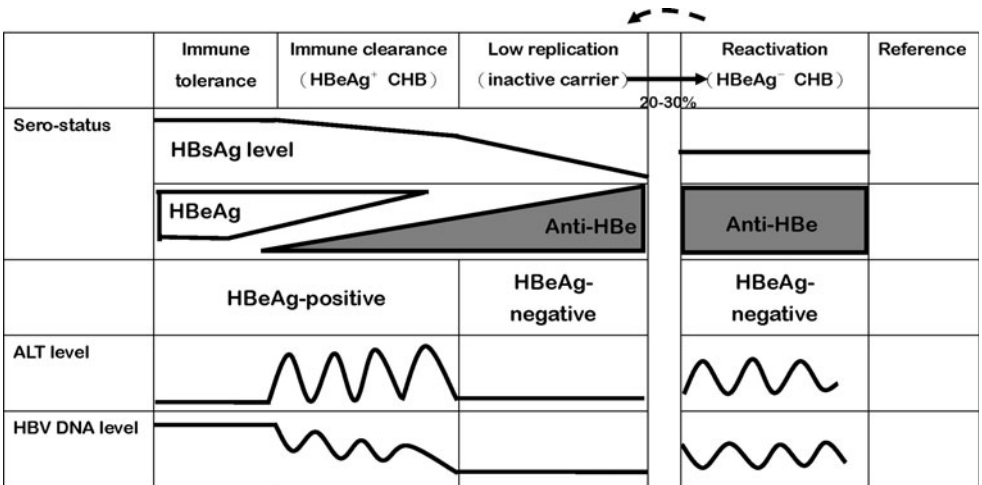

Fig. 2 HBsAg levels in different phases of chronic HBV infection. *HBeAg* hepatitis B e antigen, *CHB* chronic hepatitis B, *ALT* alanine aminotransferase, *Anti-HBe* HBe antibody

DNA occur in the inactive carrier state but that the levels are higher in the reactivation phase or HBeAg-negative hepatitis stage.

Predictive values of HBsAg and HBV DNA in clinical outcomes of chronic hepatitis B infection

Considering long-term outcomes of patients with chronic HBV infection, favorable outcomes include an inactive carrier state and loss of HBsAg, whereas adverse outcomes include HBeAg-negative hepatitis, cirrhosis and HCC.

Brunetto et al. [19] first studied whether serum HBsAg levels may contribute to diagnosis of clinical stages in HBeAg-negative HBV carriers with genotype D infection. They analyzed 209 untreated and asymptomatic carriers in Italy and found the combination of single-point quantification of HBsAg (<1000 IU/mL) and HBV-DNA (<2000 IU/mL) could identify a 3-year inactive state with 94.3 % diagnostic accuracy, 91.1 % sensitivity, 95.4 % specificity, 87.9 % positive predictive value, and 96.7 % negative predictive value. Our recent study also confirmed that a combination of HBsAg (<1000 IU/mL), normal serum ALT level, and HBV-DNA (<2000 IU/mL) could identify minimal-risk HBV carriers in Taiwanese patients with HBV genotype B or C infection (Hepatology, doi: 10.1002/hep.26041).

It is widely accepted that spontaneous clearance of HBsAg correlates with better clinical outcomes because it usually indicates disease remission [15, 20–23]. As long as there is no evidence of liver cirrhosis or HCV/HDV superinfection, and as long as the subject's age is <50 years at the time of HBsAg loss, there is a minimal risk of HCC development [20]. Previous longitudinal studies have indicated that the annual rate of HBsAg loss is approximately 0.4–2.3 %, depending on the subject's age and status of liver disease at enrolment [24–30]. However, it usually needs a large-scale cohort study with a long-term follow-up to address this issue of HBsAg loss because of its low incidence rate.

Earlier studies suggested that the HBsAg loss rate was lowest in HBeAg-positive patients, followed by that in patients with HBeAg-negative hepatitis, with the loss rate being highest in inactive carriers [15]. In a longitudinal study from Taiwan, in which a total of 1965 HBeAg-negative patients with normal ALT levels were enrolled, Chu et al. [25] reported an annual HBsAg loss rate of 1.15 %. They found that HBeAg-negative patients with sustained normal ALT levels were more likely to clear HBsAg than those with hepatitis relapse. Subsequently, a community-based large cohort study, Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV), further demonstrated that a lower HBV DNA level was the major predictor of HBsAg loss over time [28]. In adult patients with an undetectable viral load (<60 IU/mL), the annual rate of HBsAg loss was 5.76 %. Compared with patients with an HBV DNA level of ≥200000 IU/mL, the hazard ratio (HR) of HBsAg loss was 15.9 [95 % confidence interval (CI) 9.3–27.2]. Because patients with low viral loads usually have a low risk of hepatitis relapse, the association between a limited viral replication and a higher chance of HBsAg loss could explain why HBsAg loss occurs more frequently in patients with persistently normal ALT levels.

Our hospital-based cohort study, Study of E Antigen seRoClearance of Hepatitis B patients (SEARCH-B), enrolled 390 spontaneous HBeAg seroconverters and highlighted the impact of the HBsAg level at 1 year post-HBeAg seroconversion on subsequent HBsAg loss [29]. During an average follow-up period of 7.4 years, 18 patients cleared HBsAg, with an annual rate of 0.6 %. It was found that HBsAg level, compared with HBV DNA level, served as a better predictor of HBsAg loss. Even in patients with an HBV DNA level of <200 IU/mL at 1 year post-HBeAg seroconversion, an HBsAg level of <100 IU/mL remained an independent predictor of HBsAg loss. Although this study shed light on the importance of HBsAg level, the statistical power was not strong enough to fully address the relationship between HBsAg level and HBsAg loss. We therefore conducted another hospital-based cohort study, Elucidation of Risk fActors for DIsease Control or Advancement in Taiwanese hEpatitis B carriers (ERADICATE-B), and enrolled 2688 patients with chronic hepatitis B infection. We analyzed the data of 688 HBeAg-negative patients who had HBV DNA level <2000 IU/mL at baseline and were enrolled in the earlier stage of the study [30]. During a mean follow up of 11.6 years, 130 patients cleared HBsAg, with an annual rate of 1.6 %. We consistently found that HBsAg level, compared with HBV DNA level, served as a better predictor of HBsAg loss. In addition, the annual clearance rate of HBsAg reached 7 % in patients with an HBsAg level of <10 IU/mL [HR of 13.2 (95 % CI 8.1–21.5) when compared with an HBsAg level of ≥1000 IU/mL]. This large-scale study firmly confirmed the importance of HBsAg level on the development of HBsAg loss.

In addition to these cohorts, several other studies have also validated the relationship between HBsAg level and HBsAg loss (Table 1). A cohort study and a case-control study from Hong Kong showed a very similar trend: a lower HBsAg level was associated with a higher chance of HBsAg loss; HBsAg levels of 100 and 200 IU/mL, respectively, were their recommended cutoffs [31, 32]. Other studies from Taiwan, including the REVEAL-HBV cohort study, a pediatric cohort study, and a case-control study, all had very similar findings [33–35]. Although the

Table 1 Relationship between hepatitis B antigen (HBsAg) level and HBsAg loss in the literature

Country	Study design	Disease stage	Number of subjects	HBsAg cutoff (IU/mL)	Note	Reference
Taiwan	Cohort	Early HBeAg-negative stage	390	100	SEARCH-B	[29]
Hong Kong	Cohort	HBeAg-negative	103	100		[32]
Taiwan	Cohort	HBeAg-negative with hepatitis B virus (HBV) DNA level <2000 IU/mL	688	10	ERADICATE-B (partial)	[30]
Taiwan	Case–control	HBeAg-negative	46–46	200		[35]
Hong Kong	Case–control	HBeAg-negative	203–203	200		[31]
Taiwan	Cohort	Children	349	1000		[34]
Taiwan	Cohort	Including HBeAg-positive and -negative	3466	10	REVEAL-HBV	[33]

recommended cutoffs varied among the different study populations, all these studies pointed to the main finding that a lower HBsAg level was closely associated with a higher likelihood of HBsAg loss over time.

As to the prediction of disease progression, most longitudinal cohort studies have already agreed that chronic hepatitis, cirrhosis, and HCC are sequential complications of chronic hepatitis B infection [9, 12–14] and HCC is the most deadly one. Therefore, all the investigators were looking for the key factors affecting disease progression. The REVEAL-HBV study is the first cohort study to disclose that the serum HBV DNA level is a major driving force of adverse clinical outcomes [3, 36]; the authors of that study found that a higher HBV DNA level was associated with both cirrhosis and HCC development, with a dose-response relationship in adult HBV carriers. In addition, the risk started to increase when patients had HBV DNA levels of ≥2000 IU/mL. This important finding established the HBV DNA threshold level of 2000 IU/mL for treating HBV carriers and the use of a level of <2000 IU/mL for defining inactive HBV carriers [37–39].

When the assay of HBsAg quantification became available, the association between HBsAg level and HCC was firstly revealed by the ERADICATE-B cohort [40]. This cohort included 2688 Taiwanese HBV carriers who had no evidence of cirrhosis at baseline and were treatment-free during the follow-up period. At the beginning, the study demonstrated that both HBV DNA and HBsAg levels positively correlated with HCC development. When these two biomarkers were compared in predicting HCC, HBV DNA level served as a better predictor in the overall cohort. However, when the study population was limited to 1068 HBeAg-negative patients with an HBV DNA level of <2000 IU/mL, in whom HBV DNA level has little impact in predicting HCC, HBsAg level remained the only viral risk factor. More specifically, in HBeAg-negative patients with an HBV DNA level of <2000 IU/mL, there was an increased HCC risk for an HBsAg level of ≥1000 versus <1000 IU/mL with an HR of 5.4 (95 % CI 2.1–14.2). The 10-year cumulative incidence rate of HCC was 0.2 % for HBeAg-negative patients with an HBV DNA level of <2000 IU/mL plus an HBsAg level of <1000 IU/mL, and this rate was similar to the rate of non-HBV and non-HCV infected patients [41].

It is generally believed that cirrhosis usually results from an accumulation of extracellular matrix arising from liver cell injury, and HCC may subsequently emerge in the setting of cirrhosis [7, 9, 11–14]. If the correlation between HBsAg level and HCC is true, it is expected to exist consistent relationships between HBsAg level and HBeAg-negative hepatitis as well as cirrhosis development. On the basis of this hypothesis, we analyzed the 1068 HBeAg-negative patients with low viral loads from the ERADICATE-B study. An HBsAg level of ≥1000 IU/mL was noted to be consistently associated with a higher risk of HBeAg-negative hepatitis and cirrhosis. As opposed to an HBsAg level of <1000 IU/mL, the HR of HBsAg ≥1000 IU/mL was 1.4 (95 % CI 1.1–1.8) for HBeAg-negative hepatitis and 2.2 (95 % CI 1.1–4.2) for cirrhosis development (Hepatology, doi:10.1002/hep.26041).

Taking all these lines of evidence together, combining HBV DNA and HBsAg levels could be used to define "minimal-risk" patients with chronic HBV infection. The term "inactive carrier" was not used in our serial studies, because its operational criteria included persistently low levels of ALT and HBV DNA, which are very unlikely to be used in a large-scale cohort study. With snapshot HBV DNA and HBsAg levels, it is possible to identify patients with minimal risk of disease progression in our daily clinical practice. In fact, the REVEAL-HBV cohort study also reported a finding very similar to that of the ERADICATE-B cohort study: a lower HBsAg level in patients with an HBV DNA level of <2000 IU/mL was associated with a lower risk of HCC [42]. On the basis of these data, it

is believed that HBsAg level helps predict the prognosis of HBV carriers; however, the following issues need to be addressed. First, the appropriate HBsAg cutoff level needs further examination. Second, most of the data have come from Asia, and it is unclear whether this finding holds true in Western countries where genotypes A and D prevail and most patients acquire the infection later in life. If more lines of evidence can confirm the predictive value of HBsAg, HBsAg level should be included in the future development of a risk calculator or nomogram for HBV carriers and treatment guidelines for chronic hepatitis B [43, 44].

Decline of HBsAg in natural history of HBV infection

In the natural history of chronic HBV infection, declines of HBV DNA and HBsAg levels are important milestones towards good prognosis [40, 45]. Compared to the fluctuation of HBV DNA level and its remarkable decline after HBeAg seroconversion [29], HBsAg level is more stable and the decline in HBsAg level is usually slow [16, 29]. The REVEAL-HBV study indicated that HBsAg loss was preceded by seroclearance of HBV DNA [28]. A recent study from Hong Kong also showed that patients with an undetectable HBV DNA level had a more pronounced reduction of HBsAg levels than those with high HBV DNA levels [46]. Therefore, although the levels of both markers decrease over time, the seroclearance of HBV DNA usually precedes a prominent reduction of HBsAg level. In addition, previous studies have reported that reductions of HBsAg by 1 log IU/mL [16] and 0.5 log IU/mL [31] are associated with a higher chance of HBsAg loss. Taken together, these findings suggest that a rapid reduction of HBsAg usually follows the seroclearance of HBV DNA and that such a reduction of HBsAg may lead to a higher likelihood of HBsAg loss.

Of particular note is that all these longitudinal studies are coming from Asian countries. Whether these observations hold true for Western patients warrants more studies.

Clinical significance of quantitative HBsAg in chronic hepatitis B patients with antiviral therapy

Table 2 shows current treatment strategies for chronic hepatitis B [47]. The therapeutic endpoints for chronic hepatitis B include sustained suppression of HBV DNA level, normalization of serum ALT level, histologic improvement, HBeAg loss or seroconversion for HBeAg-positive patients, and, ideally, HBsAg loss or seroconversion [37–39]. Nowadays, seven approved agents for the treatment of chronic hepatitis B are as follows. Standard or pegylated interferon alpha (IFN or Peg-IFN) and five nucleos(t)ide analogues (NUCs)–lamivudine, telbivudine, entecavir, adefovir dipivoxil, and tenofovir disoproxil furamate. Although we have different agents in clinical practice, there are only two treatment strategies, immune control (sustained off-therapy response) and viral control (maintained on-treatment response).

qHBsAg and Peg-IFN therapy

qHBsAg and Peg-IFN therapy has been comprehensively reviewed elsewhere [48, 49]. In brief, on Peg-IFN treatment, sustained responders tend to show a greater HBsAg decline than non-responders. The optimal on-treatment HBsAg cutoff to predict response in HBeAg-positive patients may be 20000 IU/ml at week 12 or 24 of therapy [50, 51]; however, determination of the cutoff needs further evaluation by prospective studies. In contrast, an absence of HBsAg decline together with a <2 log reduction in HBV DNA at week 12 can serve as a futility rule in HBeAg-negative patients with HBV genotype D infection [52, 53].

qHBsAg and NUC therapy

The decline of HBsAg during NUC therapy is less pronounced than that during Peg-IFN therapy [54]. In patients receiving NUCs, the decline of HBsAg appears more apparent in HBeAg-positive patients than in HBeAg-negative patients [55]. Two large-scale clinical trials have shown that the reduction of HBsAg is pronounced within the first year of telbivudine or tenofovir treatment, and that HBsAg level remains relatively stable thereafter [55, 56]. For HBeAg-negative patients, HBsAg level does not vary significantly during NUC therapy [55]. Other studies had similar results [57–59]. Two observational studies including HBeAg-positive patients with entecavir therapy indicated that patients with HBeAg loss/seroconversion had an early decline of HBsAg levels, while those without HBeAg loss/seroconversion did not [57, 59]. Although the mechanism of HBsAg decline during NUC therapy is unclear, it may be hypothesized that the reduction of HBsAg level reflects a better degree of host immune control against the virus or even a decrease in the amount of intrahepatic cccDNA. From a conceptual viewpoint, it is known that NUC only blocks the reverse transcriptase, which diminishes HBV DNA synthesis but lacks a direct effect on cccDNA. Therefore, the observation that Peg-IFN produces a more pronounced HBsAg decline than NUCs do is reasonable, because Peg-IFN can induce apoptosis or necrosis in HBV-infected hepatocytes. On the other hand, the early

Table 2 Current treatment strategies for chronic hepatitis B

Treatment	Strategy	Goal	Duration	Effectiveness
Standard or pegylated interferon alfa	Sustained off-therapy response (immune control)	Low HBV DNA level (<2000 IU/mL) and normal alanine aminotransferase (ALT) level	Finite	Sustained response in ~30 % of patients after 48 weeks of therapy, and may increase to 50 % in those with good baseline and on-treatment factors
Nucleos(t)ide analogues (lamivudine, adefovir, telbivudine, entecavir, or tenofovir)	Maintained on-treatment response (viral control)	Undetectable HBV DNA level and normal ALT	Prolonged or indefinite	Successful suppression of HBV DNA with continued treatment without drug resistance

decline of HBsAg in patients receiving NUCs may be attributed to the restoration of the host immune response against HBV as reflected by the pretreatment ALT level. Thus, when the effect of the immune reaction diminishes, such as during the second or third year of NUC treatment, the decline of HBsAg would be less significant. However, this speculation does not totally exclude the utility of NUCs to lower HBsAg level. As we have learned the lessons from the natural history of HBV infection, achieving undetectable HBV DNA is essential for lowering the HBsAg level or even for clearing HBsAg and NUCs are characterized by their ability to inhibit HBV DNA synthesis to a very low or undetectable level. Furthermore, most studies of NUCs included patients who had been receiving the treatment for less than 5 years. Therefore, it is premature to conclude that NUCs fail to significantly reduce HBsAg levels over time.

HBsAg loss has been reported during NUC therapy in HBeAg-positive patients. In HBeAg-positive patients treated with tenofovir, the rates of HBsAg loss were 3, 6, and 8 % after 1, 2, and 3 years of therapy, respectively. Nevertheless, HBsAg loss was not observed in HBeAg-negative patients [55]. The HBsAg loss rates for HBeAg-positive patients treated with entecavir or lamivudine for 2 years were 5 and 3 %, respectively [60]. For HBeAg-positive patients receiving telbivudine and tenofovir therapy, a rapid decline of the HBsAg level during the first year of therapy was associated with a higher probability of HBsAg loss [56, 61]. Of note, the correlation between HBsAg decline and HBsAg loss in NUC users is in line with what we have observed in the natural history: the greater the decline of HBsAg, the higher is the chance of spontaneous HBsAg loss. However, most patients with NUC-induced HBsAg loss have HBV genotype A or D infection [55, 56, 60], and the clearance of HBsAg by NUCs seems very rare in Asian patients with genotype B or C infection. In other words, it may not be practical to consider HBsAg loss as the therapeutic endpoint for Asian HBV carriers. Our recent data derived from the investigation of HBV's natural history has shown that an HBsAg level of <1000 IU/mL plus an HBV DNA level of <2000 IU/mL may determine minimal-risk HBV carriers [40]. In future, we should evaluate whether a lower HBsAg level, say <1000 IU/mL at the end of NUC therapy, could serve as a therapeutic endpoint for such patients with HBV genotype B or C infection.

Perspectives and conclusions

Although we have understood more about the clinical utility of quantitative HBsAg in the management of patients with chronic HBV infection in the past years, there still exist several unmet medical needs. First, we need more data, especially cohort studies from Western countries, to confirm the findings observed in Asian patients. More importantly, we need to determine an appropriate cutoff to define minimal-risk HBV carriers, depending on how high an HCC risk we can tolerate. Second, the role of the HBsAg level in predicting HCC risk in HBV carriers with intermediate viral loads (between 2000 and 20000 IU/mL) remains to be explored. Third, the usefulness of combining HBsAg and HBV DNA levels for the risk stratification of HBeAg-negative disease needs further examination. Finally, more prospective studies are awaited to evaluate the impact of baseline and on-treatment HBsAg levels on the identification of responders and non-responders in patients receiving anti-HBV therapy, especially NUC therapy.

Conflict of interest J.H. Kao is a member of the speaker's bureau for Roche, BMS, Gilead, Novartis, and Bayer, and has received a grant from BMS. T.C. Tseng declares no potential conflicts of interest.

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