The role of quantitative hepatitis B surface antigen revisited

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Summary

In the past 10 years, there has been a lot of enthusiasm surrounding the use of serum hepatitis B surface antigen (HBsAg) quantification to predict disease activity and monitor treatment response in chronic hepatitis B. The measurement of HBsAg levels have been standardized in IU/ml, and nowadays it is almost a mandatory measurement due to the development of new antiviral treatments aiming at HBsAg seroclearance, i.e., functional cure of hepatitis B. Recently, there has been an improved understanding of the molecular virology of HBsAg, and particularly the relative roles of covalently closed circular DNA and integrated hepatitis B virus (HBV) DNA. This has shed new light on the interpretation of HBsAg levels in different phases of chronic hepatitis B. HBsAg level can assist the differentiation of immune tolerance and immune clearance in hepatitis B e antigen (HBeAg)-positive patients, and it can predict inactive disease and spontaneous HBsAg seroclearance in HBeAg-negative patients. The determination of HBsAg level is pivotal to individualize pegylated interferon (PegIFN) treatment; it is the key investigation to decide early termination of PegIFN among non-responders. Among patients treated by nucleos(t)ide analogues, responders tend to have dramatic reduction of HBsAg to low levels, which may be followed by HBsAg seroclearance. With newer data on combination treatment of PegIFN and nucleos(t)ide analogues as well as emerging new antiviral agents, HBsAg quantification is expected to become increasingly important to monitor and guide antiviral therapy for chronic hepatitis B.

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Introduction

In the last decade, there has been resurgence in research interest in hepatitis B surface antigen (HBsAg) quantification in the serum. The enthusiasm started with the observation that serum HBsAg level could possibly reflect the amount and transcriptional activity of covalently closed circular (ccc) DNA inside the hepatocytes [1,2]. Numerous studies on kinetics of serum HBsAg among untreated patients at different stages of chronic hepatitis B (CHB), and among patients treated by pegylated interferon alfa (PegIFN) and nucleos(t)ide analogues (NA) have been conducted in the subsequent years. As highlighted in a 2011 report published by a core group, in which the authors of this review article were key members, there were still a lot of unanswered questions that set hurdles for generalized usage of serum HBsAg quantification in clinical practice [3].

As new potential therapeutic targets of hepatitis B virus (HBV) have been identified in recent years, HBsAg seroclearance is now considered as the goal for functional cure of CHB [4]. As a result, serum HBsAg kinetics has become almost a routine test in clinical studies of new HBV therapeutics. In recent years, there have also been new insights from studies carried out in different parts of the world. These have further explored molecular virology and the clinical use of serum HBsAg levels to monitor natural disease progression and to predict treatment response in CHB. In view of the new information now available, and increased medical need, this review article aims to update clinicians on the indications and limitations concerning the use of serum HBsAg quantification in clinical practice and to highlight the remaining unanswered issues for future research. The suggestions are based on evidence from the published literature where possible, and on the experience and expert opinion of the authors where data is lacking.
Molecular virology of HBsAg

Virology of HBsAg production

The HBV encodes the three proteins of the HBsAg, which form the viral envelope, small (SHBsAg), middle (MHBsAg) and large (LHBsAg) (Fig. 1) [5]. These proteins are translated from two HBV sub-genomic mRNA transcripts, the preS1 mRNA and the preS2/S mRNA, in the endoplasmic reticulum (ER). HBV genomic replication occurs via reverse transcription of the pregenomic RNA (pgRNA), a greater than genome length transcript in the cytosol, thus the HBsAg ER-secretory pathway and the viral DNA replication pathway can be viewed as distinct but cross-regulated processes within the infected hepatocyte [6] (see Fig. 2). All three envelope proteins have a glycosylated form responsible for the secretion of viral particles. A common N-glycosylation site at residue asparagine sN146 is situated in loop 2 of the “a” determinant within the common S domain. The M protein also displays a N-glycosylation site at mN4 in the preS2 domain; this residue is not modified in the L protein (Fig. 1B). The virion contains a majority of S proteins and an equal amount of M and L proteins, which represent approximately one third of the viral envelope (Fig. 1C). An excess of S protein over the M and L protein is necessary for envelopment and secretion of all types of HBsAg particles [7]. The S protein exposes a low affinity-binding site for heparan sulphate proteoglycan (HSPG) which is required for infectivity (see Fig. 2). The L protein with the preS1 domain facilitates the envelopment of the core particles and includes the high affinity attachment site of HBV, the sodium taurocholate co-transporting polypeptide (NTCP) [8]. The M protein is non-essential for infection and morphogenesis but is conserved among other hepadnaviruses and binds polymerised human serum albumin [9].

The surface proteins also assemble to generate non-infectious excess sub-viral particles (SVP), approximately 22 nm in diameter with either a spherical or a long filamentous form (Fig. 1C). The SVPs can be found in great excess over virions (over 100,000 fold) in the serum of persons chronically infected with HBV (10^{13}/ml), whereas the filamentous particles are less numerous (up to 10^{11}/ml). These SVPs predominantly contain the S protein, with the spherical particles containing mainly S and M whereas filaments mainly contain a majority of S proteins with equal amounts of M and L proteins. The spherical SVPs follow the Golgi pathway for secretion whereas the filaments and virions follow section via multi-vesicular bodies and the endosomal sorting complexes required for transport.

Fig. 1. The HBV encodes the three proteins of the HBsAg, which form the viral envelope from two HBV sub-genomic mRNA transcripts, the preS1 mRNA and the preS2/S mRNA. (A) The open reading frames (ORF) of the HBV genome. (A and D) The overlapping relationship between the envelope ORF and the HBV polymerase ORF is highlighted. (B) Post translational modification of preS1, preS2 and S. Symbols: ◆ myristylation; ● glycosylation. (C) The arrangement of L, M and S into virions and sub-viral particles.
ESCRT) complex. The role of the SVPs in the pathogenesis of CHB is still unclear; they may act as immunological decoys for the host immune system by blocking neutralising antibodies to HBsAg (anti-HBs), thereby promoting virus spread and persistence in the infected host [7].

Role of episomal integrated HBV DNA

Productive replication of HBV, a DNA virus that replicates via reverse transcription, is driven from its transcriptional template, the cccDNA, which is found in the nucleus as a viral minichromosome [10,11]. Transcription from this minichromosome generates all the mRNAs needed for HBV replication including SVP production. HBsAg may also be produced from HBV DNA integrated into the host genome (Fig. 2). Although viral integration is not required for normal productive replication, integration of HBV DNA occurs illegitimately through recombination mechanisms using host enzymes acting on the double-stranded linear (DSL) DNA form of HBV [12]. The DSL DNA replicative intermediate is generated as a consequence of the failure to translocate the RNA primer needed to prime plus-stranded DNA synthesis of the HBV genome [13]. The DR-1/DR-2 regions of the viral genome are preferential sites for integration [14], but integrated sequences cannot provide a template for productive viral replication since the complete genome is not present. However, the open reading frame (ORF) of the S gene with its regulatory elements are often still present in integrated sequences, are intact and so, HBsAg can be produced. Thus, two sources of HBsAg can be identified: cccDNA derived and integrated DNA derived (Fig. 2). This has significance, not only in defining cure end-points such as functional or complete [15] but also in attempting to correlate

Fig. 2. A schematic diagram of the HBV life cycle. The following processes are highlighted: (a) Virus (SL-10) attachment via NTCP and HSPG receptors; (b) the nuclear reservoir of covalently closed circular (ccc) DNA, which is the transcriptional template for the virus; (c) the HBsAg secretory pathways; (d) the viral replication pathways; and (e) the major HBV DNA integration pathway from DSL-DNA. Nucleos(t)ide analogue therapy, by targeting the HBV reverse transcriptase (RT), selectively inhibits virion production, but does not reduce HBsAg levels, as levels of cccDNA remain unaltered. The spherical sub-viral particles of HBsAg are produced and secreted via the ER-Golgi complex, whilst virions and filaments are formed by budding from multivesicular bodies (MVB). In contrast to NA therapies, the IFN-based strategies can non-cytolytically clear hepatocytes of HBV cccDNA infection, resulting in reductions in translated and secreted HBsAg. Protective antibodies generated from vaccination block the binding of the ‘a’ determinant to the HSPG receptor.
HBsAg levels in serum to particular replicative markers in the liver.

**Differences in HBeAg-positive and HBeAg-negative infection**

In CHB, viral integration seems to occur early in infection, but at very low levels during the HBeAg-positive phase of the disease. Recent studies in chronically infected chimpanzees have demonstrated that there is a dramatic increase in the number of integration events in animals in the HBeAg-negative phase compared to the HBeAg-positive phase [16], so much so that >90% of the mRNA in livers of HBeAg-negative chimpanzees was derived from integrated HBV sequences, with only 10% derived from the HBV minichromosome [16]. This data would seem to add another layer of challenge to achieving a complete cure for CHB, but does account for the lack of correlation between HBsAg levels in the serum and intrahepatic cccDNA levels in patients with HBeAg-negative CHB [17–19]. Interestingly, a positive correlation has been noted between HBsAg titres and serum HBV DNA, and liver cccDNA in most studies of HBeAg-positive patients [18].

**Antiviral drug resistance and HBsAg quantification**

The genome of the HBV is a circular double-stranded DNA with four overlapping but frame shifted ORFs (Fig. 1A). The envelope ORF completely overlaps the polymerase gene, with the result that point mutations in the polymerase ORF selected by nucleos(t)ide analogue (NA) therapy may result in important changes, including stop codons, in the envelope protein (Fig. 1D). The substitution rtA181T and rtM204I associated with NA resistance and multi-drug resistance, results in stop codons sw172 and sw196 in the overlapping S-ORF, respectively [20]. Such stop codons do result in reduced secretion of HBsAg, and also intracellular retention with increased risk of cellular transformation [21].

**Technology of HBsAg quantification**

The first report on standardized HBsAg quantification in weight units per volume was more than 40 years ago [22]. During recent years a number of quantitative assays for HBsAg have now been developed fulfilling the prerequisites of a biomarker: reproducibility, automated quantification, and a relatively low cost and standardization using IU/ml. It is important to appreciate that these assays detect all three forms of circulating HBsAg, the Architect QT assay (Abbott Laboratories), the Elecsys HBsAg II Quant assay (Roche Diagnostic) and Diasorin Liaison XL. All three assays have been extensively evaluated and closely correlate with each other [23–25]. The dynamic range of the Architect assay is from 0.05 to 250 IU/ml, for the Elecsys II assay the range is 0.05 to 130 IU/ml, while the Liaison XL quantitation range is set from 0.03 to 150 IU/ml [23]; all three systems have automatic on board dilution, typically 1:400, thereby increasing the upper limit of detection to over 50,000 IU/ml.

Importantly, several factors may influence the results of HBsAg quantification. For example, HBsAg quantitation may become inaccurate or misleading if HBsAg escape mutants are predominant [26]. In addition, HBsAg is quite often partially masked in immune complexes, which could influence the results [27].

As mentioned above, the current commercially available quantitative HBsAg assays are unable to differentiate between the three HBs protein subtypes. Some studies are underway to analyze if quantification of the HBs proteins are useful as biomarkers and whether they can provide any information beyond that provided by a commercial HBsAg assay. So far these assays are based on in-house quantitative enzyme-linked immunosorbent assay or Western blot analysis using monoclonal antibodies directed against the S domain (to determine total HBs protein levels), the preS1 domain (to determine HBV large surface protein (LHBs) protein levels) and the N-glycosylated preS2 domain (to determine HBV middle surface protein (MHBs) protein levels) [28].

**Natural history of HBV infection**

HBsAg production, that follows cellular pathways distinct from viral replication, depends, at least in part, on constitutive viral features, such as genotype and quasispecies [29–33]. Accordingly, in vitro HBsAg production differs among HBV genotypes and in vivo HBsAg serum levels are higher in genotype A (A2 and A1) than genotype B, C and D infected individuals [30–32]. In addition, it was shown that preS/S mutants may impact on HBsAg production as they are negatively correlated with HBsAg serum levels [33]. Finally, both quantitative and qualitative production of HBsAg are modulated by the interplay between virus and host immune response and HBsAg serum levels are inversely correlated with the immune control of HBV: the higher control, the lower HBsAg [34,35]. Therefore, only the combined quantification of HBsAg and HBV DNA can contribute to pinpoint more accurately the single HBV carrier within the highly dynamic phases of
chronic HBV infection, provided that all variables interfering with HBsAg production are carefully considered.

**Acute HBV infection**

In contrast to chronic HBV infection, HBsAg dynamics seem to be strongly correlated with the decrease of HBV DNA in the acute phase of HBV infection [34]. Thus, HBsAg quantification is also useful to monitor and predict the outcome of acute hepatitis B as already in reported in the 1970s [36].

**HBeAg-positive carriers**

HBsAg serum levels are higher in the high replicative non-inflammatory (formerly immune-tolerant) than in the immune clearance phase (4.5–5 vs. 3.7–4.3 log$_{10}$ IU/ml) [34,35]. High, stable HBsAg (~5 log$_{10}$ IU/ml) and HBV DNA (>8 log$_{10}$ IU/ml) serum levels were shown to hallmark the immune-tolerant phase in HBeAg-positive Asian carriers [37]. Accordingly, HBsAg serum levels ≥25,000 IU/ml and normal ALT (<2 x ULN) showed 86.4% sensitivity, 75% specificity and 92.7% positive predictive value (PPV) to predict low fibrosis (<F1) in 140 Chinese HBeAg-positive carriers [38]. A French study, including all HBV genotypes, confirmed that HBsAg is significantly higher in F0–F1 than F2–F4 patients (4.63 ± 0.58 vs. 3.84 ± 1.01 log$_{10}$ IU/ml, p <0.001) and proposed a 3.85 log$_{10}$ IU/ml threshold to identify moderate-severe fibrosis (100%-sensitivity, 85%-specificity and 100%-negative predictive value (NPV)) in genotype C patients [39]. Similar results were obtained in 197 Chinese patients [40], whereas a higher threshold was suggested by a preliminary report in genotype A/D patients [41].

HBsAg does not predict spontaneous HBeAg seroconversion and thereafter its decline is very slow in most of the cases [37,42]. Long-term follow-up studies of HBsAg kinetics are missing, particularly in patients progressing to HBeAg-negative CHB and carriers with transition to low viremic/inactive infection. Therefore, at present, in the early phase of HBeAg seroconversion, HBsAg serum levels do not help to distinguish inactive carriers from CHB patients, with the exception of the carriers whose HBsAg levels drop to <1000 IU/ml within 1-year from HBeAg seroconversion in whom the chance of HBsAg clearance within 6 years is high [43,44].

**HBeAg-negative carriers**

The spectrum of clinical conditions associated with HBeAg-negative phase is wide, ranging from benign, non-progressive inactive infection to CHB with high risk of cirrhosis and hepatocellular carcinoma (HCC). Therefore, the early identification and treatment of CHB is a major clinical challenge, however, HBeAg-negative CHB may present major fluctuations of both serum HBV DNA and ALT with temporary remissions that mimic inactive HBV infection [45]. The evidence that HBsAg serum levels are significantly lower in inactive carriers (IC) than CHB patients [34,35] prompted studies to investigate the role of HBsAg quantification. Independent from the heterogeneous cohorts, a common finding was that serum HBsAg levels contributes to a better diagnostic and prognostic definition of low viremic carriers. In genotype D inactive infection (HBV DNA persistently <2000 IU/ml at 1-year monthly follow-up), baseline HBsAg levels were significantly lower than in CHB patients; the combined single-point quantification of HBsAg (<1000 IU/ml) and HBV DNA (<2000 IU/ml) showed 94.3% diagnostic accuracy and 87.9% PPV in the identification of inactive carrier [46]. By using the same thresholds in HBeAg-negative, genotype B and C infected carriers of the REVEAL-cohort the diagnostic accuracy was 78% and the PPV 83% [47]. In addition, the overall REVEAL-cohort baseline serum HBsAg levels were significantly associated with the cumulative risk for disease progression (4.8, 8.8 and 16.2% for cirrhosis, 1.4, 4.5 and 9.2% for HCC with HBsAg <100, 100–999 and >1000 IU/ml, respectively). However, after stratification for HBeAg and viral load, the findings hold true only for HBeAg-negative carriers with HBV DNA <10$^5$ log copies/ml [48]. In addition, the study of 1068 HBeAg-negative patients with baseline-HBV DNA <2000 IU/ml showed a yearly incidence of HBeAg-negative-CHB of 2%, that was reduced to 1.1% in those with HBsAg <1000 IU/ml and normal ALT [49]. These carriers showed also significantly lower progression rates to cirrhosis and HCC as compared to those with HBsAg ≥1000 IU/ml [HCC HR:13 (95% CI 4.6–37, p <0.001)] [50]. In absence of a tight follow-up a stringent (98% specificity, 97% PPV) inactive carrier prediction can be achieved by combining HBsAg <100 IU/ml to HBV DNA <2000 IU/ml, but sensitivity decreases significantly (35%) [51]. In fact, very low serum HBsAg levels (<100 IU/ml) identify those IC with a high probability of spontaneous HBsAg clearance [51–54].

A significant correlation between serum HBsAg and fibrosis was not reported in HBeAg-negative patients [39], however several studies showed a trend for lower HBsAg levels in cirrhotics. This is possibly because of a viral quasispecies with prevalent preS/S variants (selected during the long lasting immuno-elimination phase) [33,46,55]. In conclusion, HBsAg serum levels are useful to optimize the management of the HBsAg carriers provided that they are combined with a careful evaluation of the virologic and clinical settings: the lower the best in low viral replication, but the lower the worst in long lasting florid replication. Thus, HBsAg serum levels contribute to better define the “virologic phase of the infection” without providing direct information on liver disease, that has to be staged
by serum biochemistry and liver imaging. Discrepant findings (i.e., a virologic profile of inactive carrier, but elevated liver stiffness values) should prompt further investigation to rule out either an intermittent viral replication responsible for CHB or a liver disease of other etiology.

**Pegylated interferon alfa therapy**

Several studies have shown that the change of serum HBsAg levels during PegIFN therapy mimics the change of both intrahepatic cccDNA and intrahepatic HBsAg, suggesting that a decline in serum HBsAg levels is associated with the induction of an effective anti-HBV immune response [2,56–58]. Therefore, HBsAg levels are a valuable tool for the monitoring of patients treated with PegIFN.

**HBeAg-positive patients**

Several studies have shown that PegIFN induced a pronounced decline in serum HBsAg level that was sustained during off-treatment follow-up [59,60]. The degree of HBsAg decline is associated with an active pre-existing immune response as reflected by elevated serum ALT levels and elevated levels of IFN gamma inducible protein-10 (IP-10, CXCL-10) [61], with presence of only wild-type HBV before initiation of PegIFN [62], and with the infecting HBV genotype. Patients with genotype A achieved the most pronounced HBsAg decline and patients with HBV genotype D the least [63].

Since serum HBsAg decline during PegIFN therapy is largely confined to patients who achieve a response, monitoring of HBsAg levels help distinguish patients likely to achieve a response from those who will not. In European patients predominately infected with HBV genotype A or D, failure to achieve a decline of HBsAg levels by week 12 of PegIFN therapy was associated with a very low chance of HBsAg levels to achieve a sustained response [69–71]. Rijckborst et al. performed a post hoc analysis of a randomized trial that included PegIFN treated patients, of whom 85% was infected with HBV genotype D. They showed that not only HBsAg levels, but also HBV DNA levels are important in the prediction of response. When combining both serum markers, the prediction of treatment failure could be improved considerably when compared to either marker alone. It was shown that when patients failed to achieve an HBsAg decline by week 12, and also failed to achieve a decline of >2 log IU/ml of HBV DNA, they had no chance of achieving a sustained response (HBV DNA <2000 IU/ml and normal ALT at 6 months post-treatment). With this so-called PARC stopping rule, a NPV of 100% was achieved, and it was shown that 20% of patients could be stopped at week 12 using this rule [70]. The findings from the original study were subsequently validated in two large independent trials, in which patients were treated for either 48 or 96 weeks with PegIFN [72,73]. The validation cohort enrolled only few non-D genotype patients, but an NPV of 95% was observed among this subset [72]. Despite the excellent performance of the PARC stopping rule in both the original and validation studies, it should be appreciated that only few non-D genotype patients have so far been analyzed. As in HBeAg-positives, it has been shown that early on-treatment HBsAg kinetics differ among HBV genotypes [32]. These results may have implications for the use of the current prediction rules, as they raise the question whether prediction rules should be genotype specific, especially with regard to patients infected with HBV genotypes A or C.

Given the high rate of post-treatment relapse despite undetectable HBV DNA at the end of PegIFN therapy, the relationship between end of treatment

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**Key point**

HBsAg level is the key on-treatment serum marker to determine stopping rules for poor responders to pegylated interferon therapy.
HBsAg levels and sustained response has been assessed. HBsAg levels <10 IU/ml at the end of PegIFN treatment had a 52% probability of HBsAg clearance during long-term follow-up [69]. In addition to these findings, it was shown that for long-term virologic response at 5 years post-treatment, high PPVs could be obtained by using HBV genotype specific end of treatment HBsAg level cutoffs. Using these end of treatment HBsAg levels for HBV genotype A (<400 IU/ml), B (<50 IU/ml), C (<75 IU/ml) and D (<1000 IU/ml) showed to achieve PPVs of 75%, 47%, 71% and 75%, for long-term virologic response, respectively [32].

Therapy with nucleos(t)ide analogues

The aim of NA treatment is maintained viral suppression during treatment [66]. The optimal endpoint and the only situation where NA treatment can be safely discontinued is HBsAg loss, which is a rare event [74]. In HBeAg-positive patients, discontinuation of NA treatment 12 months after HBeAg seroconversion can be recommended [66] but relapse is not infrequently observed [75]. HBsAg levels may help to predict who may achieve HBsAg loss in the long-term and HBsAg may also be an interesting marker to predict HBV rebound after stopping NA treatment.

General trend of HBsAg decline during nucleos(t)ide analogue therapy

HBsAg decline during NA therapy is much slower and less pronounced compared to PegIFN treatment, although NA are much more potent on HBV DNA suppression [69,76,77]. NAs inhibit only the reverse transcription of the pgRNA but do not target the cccDNA directly. Thus, changes at transcriptional levels, particularly in the HBsAg secretory pathway, are not expected. On the other hand, IFN has both direct antiviral and immune mediated effects. It is likely that the immune modulation by IFN leads to a more dramatic decline in HBsAg production and secretion. Based on HBsAg kinetics, some authors estimated that the median time to HBsAg loss in NA treated patients could be far more than 30 years [78,79]. The rate of HBsAg decline is higher in HBeAg-positive patients vs. HBeAg-negative patients [79–81]. The faster decline in HBeAg-positive patients, especially in the first year of NA therapy [80,82], may be explained by the NA effect on HBV DNA containing virions. In addition, HBeAg-negative patients may have higher amounts of integrated HBV DNA, which can still lead to HBsAg production, as discussed above. Although HBsAg decline is very slow, there are individual differences [83]. Stronger HBsAg decline during NA therapy was associated with higher pre-treatment ALT [79] or IP-10 (CXCL-10) level [83,84] confirming that immune responses are required for HBsAg decline and HBV clearance. So far there is no evidence that different NA or even combinations of NA have different effects on HBsAg kinetics [81,83]. It may be more likely that inter-current events such as infections which may induce cytokines leading to HBsAg decline. Just recently, it has been shown that IFN gamma or tumor necrosis factor alfa can reduce cccDNA without cytolysis [85].

HBeAg-positive patients

In HBeAg-positive patients it would be interesting if HBsAg level could predict HBsAg loss or sustained immune control after HBeAg seroconversion. Wursthorn et al. showed that a rapid HBsAg decline of more than 1 log after 1 year of treatment with telbivudine was predictive for HBsAg loss [86]. Supporting the aforementioned hypothesis, HBsAg decline was associated with markedly enhanced antiviral T cell reactivity. Similar data have been documented in patients treated with tenofovir or entecavir. In patients treated with tenofovir, a reduction in HBsAg level of at least 1 log by week 12 or 24 were predictive for HBsAg loss with a positive predictive value of up to 45% and a NPV of up to 97% [87]. It appears that HBeAg-positive patients with stronger HBsAg decline are more likely to achieve HBeAg seroconversion [81,88] but this was not observed in all studies [89,90]. There is less data if HBsAg level can predict off-treatment response after HBeAg seroconversion. For example, in a small study with 11 HBeAg-positive patients who were treated with telbivudine for 2 years, HBsAg <100 IU/ml at the end of treatment predicted for sustained response (defined as undetectable HBV DNA, normal ALT and HBeAg seroconversion) for 2 years after stopping treatment [91].

HBeAg-negative patients

The predictive value of HBsAg levels and interpretation of HBsAg kinetics is more difficult in HBeAg-negative patients [88,92]. HBSAg negative patients may have higher amounts of integrated HBV DNA, which can be a source of HBsAg as discussed above. Thus, the relative effect of NA on the replenishment of cccDNA is lower. In addition, non-cytolytic immune responses may have effect on cccDNA degradation [85] but not on the cell and integrated DNA. Thus, HBeAg-negative patients may have different HBsAg kinetics to cytokines (i.e., IFN treatment) or non-cytolytic immune responses during the natural course of HBV infection or NA therapy compared with HBeAg-positive patients.

Another important fact for the interpretation of HBsAg levels is that some HBeAg-negative patients with early HBsAg decline during NA therapy might have just changed the phase of HBV infection (i.e., transition from immune clearance phase to HBeAg-negative hepatitis) [83], which is associated with lower HBsAg levels [34,35]. Thus, it may represent
an already ongoing natural HBsAg decline in some patients.

HBsAg levels in HBeAg-negative patients may be useful for the decision if treatment cessation is an option. Because of the aforementioned long-term treatment with NAs, efforts have been made to identify possible stop points for NA treatment. Hadziyannis et al. showed in HBeAg-negative infection with normalized ALT and suppressed HBV DNA after treatment with adeovir for 4–5 years, the withdrawal of antiviral therapy led to a sustained off-treatment HBV DNA suppression in 55% of patients and even subsequent HBsAg loss in 39%. Lower HBsAg levels at end of treatment were predictive for later HBsAg loss [93]. Again, this supports the idea that immune responses are important for the long-term control of HBV infection. A systematic review of several studies, most retrospective suggest that some patients with decreasing HBsAg levels or lower HBsAg levels (<100 IU/ml) are able to maintain control of HBV after discontinuation of long-term NA therapy [94]. In addition, a consolidation therapy of >3 years before stopping NA seem to be important as well [94]. However, stopping NA might be more complex than anticipated. Some studies suggest that HBV DNA rebounds shortly after NA cessation in most cases [93,95]. This may induce ALT flares [93,95] indicating immune responses, which may be the key for subsequent immune control and HBsAg loss [96]. Therefore, stopping NA is not at all recommended in patients with cirrhosis.

Combination therapy of pegylated interferon alfa and nucleos(t)ide analogues

Since PegIFN and NA control HBV by different mechanisms, it is logical to try combining the two for better efficacy [97]. Although the combination of PegIFN plus lamivudine failed to improve sustained virological response over PegIFN alone [98–102], a recent multicenter trial (study 149) showed that HBsAg seroclearance could be achieved in 9.1% of patients receiving a combination of PegIFN and tenofovir for 48 weeks, compared to 2.8% of those receiving PegIFN monotherapy and none of those receiving tenofovir [92].

The target of combination treatment is off-treatment sustained response and/or HBsAg seroclearance. The predictive value of HBsAg in patients receiving PegIFN and lamivudine is similar to that in patients on PegIFN monotherapy as both treatments result in similar degree of sustained response [2,59]. In the study 149 described above [103], on-treatment HBsAg level could also predict HBsAg seroclearance in patients receiving PegIFN and tenofovir for 48 weeks [104]. Patients with a 1 log decline or HBsAg <100 IU/ml at week 12 had 43% and 50% chance of losing HBsAg at week 72, respectively; the corresponding NPVs were 97% and 95%, respectively. Patients failing to meet these criteria may stop PegIFN and continue tenofovir.

Likewise, HBsAg appears useful in patients switching from NA to PegIFN. In HBeAg-positive patients switching from entecavir to PegIFN for 48 weeks in the OSST trial, HBsAg seroclearance occurred in 20% of those with baseline HBsAg <1500 IU/ml and 2% of those ≥1500 IU/ml [105]. HBsAg at week 12 of PegIFN treatment also predicted HBsAg seroclearance (78% if <200 IU/ml vs. 1% if ≥200 IU/ml). The high NPV of HBsAg level has been validated in the year 1 analysis of the NEW SWITCH study, which tested the use of PegIFN for 48 or 96 weeks in patients achieving HBsAg loss with NA [106]. In other words, patients with high HBsAg while on NA treatment should not switch to PegIFN, and on-treatment HBsAg level can serve as a stopping rule. It should be highlighted that the prediction rules were only based on on-treatment or end of treatment HBsAg seroclearance data. In the OSST trial, only a subset of PegIFN-treated patients underwent post-treatment follow-up for 1 year [107]. Although the rate of HBeAg seroconversion continued to rise, 1 of 7 patients with HBsAg seroclearance relapsed. The robustness of baseline and on-treatment HBsAg level to predict long-term virological response warrants further study.

Co-infections

HBV/HCV

The virological patterns in HBV/HCV co-infection are widely divergent and have dynamic profiles. In most cases HCV is the dominating virus [108]. One study suggest that HBsAg levels are lower in HBV/HCV patients with HCV dominance compared to active HBV mono-infection and comparable to low replicative HBsAg carriers [109]. Higher IP-10 (CXCL-10) levels in HCV dominant patients might help to explain why HCV suppresses HBV replication and also HBsAg production [109]. This may be relevant because treatment induced elimination of HCV can lead to rebound of HBV replication in some patients [110,111]. Further studies need to evaluate if HBsAg levels can predict the risk of HBV reactivation after HCV clearance.

HBV/HIV

The situation in HBV/HIV co-infected patients is different to HBV/HCV. Patients with HIV/HBV co-infection appear to have higher HBsAg levels compared with HBV mono-infected patients [112]. HBsAg levels seem to correlate negatively with the CD4+ T cell count, reflecting the importance of immune responses for the control of HBV and the level of HBsAg [112]. Some studies evaluated HBsAg kinetics during tenofovir based antiretroviral therapy (ART) and showed that similarly to HBV mono-infection,
HBsAg decline is slow [113,114] and HBeAg-positive patients experience stronger HBsAg decrease compared to HBeAg-negative patients [113]. HBsAg decline, which was predictive for HBsAg seroclearance correlated with an increased CD4+ T cell count, underlining the importance of immune restoration for HBV clearance [113,115]. Low pre-treatment HBsAg levels are also predictive for HBsAg seroclearance [114], especially in HBeAg-negative patients [116]. The lower baseline HBsAg may reflect the better immune status as mentioned above.

HBV/HDV

Hepatitis delta virus (HDV) infection only occurs as HBV co-infection or superinfection to an already existing HBV infection because HDV requires the HBsAg for entry, envelopment and secretion and consequently complete replication and transmission [117]. In most cases HDV suppresses HBV replication [118,119]. However, HBsAg levels appear to be higher or at least similar in HDV co-infected patients despite lower levels of HBV DNA, [118,119]. As of now, only IFN or PegIFN treatment has proved to achieve a virological response in HDV patients with sustained HDV RNA clearance in about 25% of patients [120]. However, late relapse can occur during longer follow-up of up to five years [121]. Thus, HBsAg loss is the most important goal for HDV infection [66]. For current treatment of the HBV/HDV co-infection with PegIFN, usually HDV RNA is monitored. So far there is no good positive or NPV to individualize treatment duration. The role of HBsAg in hepatitis delta infection remains yet to be determined. Monitoring of HBsAg levels in addition to HDV RNA in patients with chronic delta hepatitis may provide more insight during treatment and may help to guide therapy [122], i.e., to prolong treatment until HBsAg loss in those who show a decrease during treatment [123]. However, so far no stopping rules have been established.

Possible roles in future therapy

The ultimate goal for patient management should be to achieve “hepatitis B cure” after a finite duration of treatment; since a “complete cure” defined by elimination of the virus and all its replicative intermediates, appears unrealistic at present, an interim “functional cure”, which has been defined as sustained or durable HBsAg loss preferably with anti-HBs seroconversion and undetectable serum HBV DNA allowing treatment cessation [15], would appear more achievable. Most investigators are of the opinion that a multi-step approach to “hepatitis B cure” is required. This could be achieved with a depletion or silencing of the pool of cccDNA molecules and broad activation of antiviral immunity in order to overcome adaptive T cell/B-cell exhaustion [15]. This multi-step approach has not really addressed the issue of integrated HBV DNA, especially if serum HBsAg negativity is one of the clinical trial treatment end-points. The main virological targets under investigation [124] include HBV entry (NTCP-blocking), HBV transcription-translation inhibitors (siRNA), HBx inhibitors (cccDNA epigenetic regulation and silencing), blocking core/nucleocapsid assembly (inhibition of genomic replication) and cccDNA depletion (CRISPR/Cas 9; APOBEC 3A/3B) (Fig. 2). Approaches to enhance the immune response to viral antigens include therapeutic vaccines, Toll agonists, checkpoint inhibitors such as PD-1/PD-L1 and activation of APOBEC 3A/3B pathways [15]. Several of these approaches will require the development of new assays to measure the direct impact of the particular therapy on viral replication and pathogenesis, such as the capsid/nucleocapsid assembly inhibitors and the molecular approaches to cccDNA depletion. However, most approaches can still be assessed by measurement of serum HBsAg and HBV DNA levels, but in many cases may only provide an indirect measure of antiviral activity and/or host recovery. Blocking of each of the targets discussed above will have different effects on treatment end-points depending on the particular phase of CHB, reflecting the changes in the viral lifecycle across the four major phases.

Recommendations

HBsAg quantification is not a substitute for HBV DNA. It has its unique meaning, but it has to be interpreted and used with HBV DNA and other viral tests in different clinical settings.

Assessment of untreated patients

In HBeAg-positive patients, HBV DNA and HBsAg levels are usually high. The key clinical questions are severities of liver fibrosis and hepatic necroinflammation, which determine the need of antiviral therapy and risk of HCC. Although lower HBsAg level tends to associate with immune clearance and more advanced liver fibrosis, its clinical use is limited and cannot replace biochemical and histologic assessments.

In HBeAg-negative patients, patients with HBV DNA >2000 IU/ml and patients with advanced liver fibrosis have high risk of disease progression and HCC; antiviral therapy has to be considered but HBsAg has limited role in the decision on the need of treatment. However, among patients with HBV DNA <2000 IU/ml and without liver fibrosis, HBsAg <1000 IU/ml signifies inactive carrier state with low HCC risk and HBsAg <100 IU/ml is associated with a high chance of spontaneous HBsAg seroclearance. In resource-limited settings, low risk patients, i.e., young patients with low HBsAg level with no
evidence of liver cirrhosis, can be cared by primary physicians and may not need HCC surveillance.

Monitoring of pegylated interferon alfa treatment

As PegIFN has a relatively low sustained response rate and has numerous side effects, one should individualize this treatment to patients with the highest chance to respond and stop it as soon as possible if the chance of response is dismal. Although a lower HBsAg level is associated with better response in treatment naïve patients, baseline HBsAg has limited role in selecting patients to treat in addition to other clinical parameters such as HBV genotype, HBV DNA and ALT level. On the other hand, on-treatment HBsAg levels at week 12 and week 24 have high NPVs for response, and are useful to serve as stopping rules for the non-responders (Table 1). Among patients with low or intermediate level of on-treatment HBsAg, there is insufficient evidence to suggest extension of PegIFN treatment or addition of NA can improve treatment response.

Monitoring of nucleos(t)ide analogue treatment

HBV DNA is the key marker to monitor for efficacy and adherence of NA treatment. Although a small proportion of patients have HBsAg level dropped very fast in the first year of NA treatment, most patients have HBsAg level declined very slowly. Monitoring of HBsAg level can give an estimate on the duration of NA treatment needed to achieve HBsAg seroclearance, but monitoring more frequent than 6–12 months is unnecessary. HBsAg levels may be useful to predict HBV reactivation or sustained response after cessation of NA therapy. HBsAg <100 IU/ml seem to be predictive for sustained response but so far there are no established cut-offs. Currently, HBsAg seroclearance is still the acceptable endpoint to stop NA in HBeAg-negative patients.

Combination therapy

In general, combination therapy, either PegIFN with NA or NA with NA, is not a recommended strategy for most CHB patients. If tenofovir and PegIFN combination treatment is used, week 12 HBsAg decline of <1 log can be used as the stopping rule for PegIFN according to the results of a global randomized controlled trial.

Among patients on long-term NA treatment, there is increasing interest to start PegIFN to achieve a functional cure of HBsAg seroclearance. Preliminary data from China suggests HBeAg-positive patients who have HBeAg loss with low HBsAg level (<1,500 IU/ml) on NA have higher chance of HBsAg seroclearance after switching to PegIFN. More data including different HBV genotypes is needed to validate this observation.

Key point

Monitoring of HBsAg level during nucleos(t)ide analogue treatment can give an estimate on the duration of treatment needed to achieve HBsAg seroclearance and predict sustained response after cessation of therapy.

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<th>Natural course</th>
<th>Treatment with PegIFN</th>
<th>Treatment with NA</th>
<th>Treatment with PegIFN and NA</th>
<th>Co-infections</th>
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<td>HBeAg positive</td>
<td>HBeAg &lt;1500 IU/ml at week 12 corresponds to 57% PPV for HBeAg seroconversion and 17.6% HBsAg clearance</td>
<td>No decline (any decline) of HBsAg at week 12 has a high NP for response (HBeAg loss and HBV DNA &lt;2000 IU/ml at 24 weeks post-treatment). Response &lt;5% (genotypes A and D)</td>
<td>HBsAg decline of &lt;1 log from baseline to week 12 is associated with high NPV for HBsAg loss</td>
<td>HBV/HCV: Lower HBsAg in HCV dominant patients</td>
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<tr>
<td>HBeAg negative</td>
<td>HBeAg &lt;100 IU/ml is probably associated with spontaneous HBsAg clearance</td>
<td>No HBeAg decline (any decline) and &lt;2 log decline of HBV DNA showed a NPV of 100% for non-response in genotype D patients</td>
<td>HBsAg decline &gt;1 log after 12–48 weeks has been associated with HBsAg loss</td>
<td>HBV/HIV: Higher HBsAg levels compared to mono-infection. Association with CD4 T-cell count. HBsAg decline during ART is associated with CD4 T-cell count</td>
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<td>HBsAg &gt;25,000 IU/ml &gt;90% PPV for minimal liver fibrosis &lt;F1</td>
<td>HBsAg &lt;100 IU/ml is associated with lower risk for HCC</td>
<td>Very slow decline of HBsAg. Low HBsAg levels (&lt;100 IU/ml) may predict off-treatment response after cessation of NA (after consolidation therapy &gt;3 years)</td>
<td>Low HBsAg levels (&lt;100 IU/ml) may predict off-treatment response after cessation of NA (after consolidation therapy &gt;3 years)</td>
<td>HBV/HDV: Relative high HBsAg level. HBsAg level may be useful to guide IFN therapy</td>
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Review

Co-infections

For HBV co-infections, the role of HBsAg remains imprecise despite a larger number of studies with an overall smaller number of patients. However, HBsAg levels may help to further understand the viral interactions and improve the management of HBV co-infections. Further studies are required.

Future therapies

Most strategies aiming for functional cure of HBV infection include combination therapy with NA. Thus, HBsAg is essential to monitor the response to new therapeutic concepts. Besides HBsAg loss, the level of HBsAg decline or certain cut-offs could serve as secondary end-points for early clinical trials. However, the exact decline or level of HBsAg that can clearly give a go or no-go signal for a new HBV therapy is uncertain.

Conflict of interest

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Authors’ contributions

HLYC was responsible for planning and administrative support. All authors (MC, VWSW, MB, SL, HLJ and HLYC) were involved in the writing of the manuscript. SL was responsible for drafting of the manuscript. MC and HLYC were involved in final editing of the manuscript.

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