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SUPPLEMENT ARTICLE

The role of quantitative hepatitis B serology in the natural history and management of chronic hepatitis B

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Abstract Chronic hepatitis B (CHB) remains a serious clinical problem worldwide. Advances in molecular technology have enabled the development of sensitive assays for the detection and quantification of hepatitis B virus (HBV) nucleic acid and demonstrated a positive correlation between serum HBV DNA levels and disease progression. Assessment of specific serologic and virologic factors also plays a pivotal role in the diagnosis and effective management of individuals with CHB. Recent development of quantitative assays for intrahepatic HBV replicative intermediates, as well as hepatitis B e antigen and hepatitis B surface antigen, has spurred investigation into the relationship between these factors and response to antiviral therapy and disease progression. Recent findings from preclinical and clinical investigations indicate that these factors may have promise in identifying patients likely to respond to treatment. Additional work is needed to standardize and validate these assays before they can be considered to be of true diagnostic value. Further evaluation is needed to decide which will have the greatest clinical applicability.

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Introduction

In the era of molecular diagnostics, significant progress has been made in the understanding of the lifecycle, clinical course, and pathogenesis of the hepatitis B virus (HBV). This has been facilitated by the improved sensitivity of HBV viral load assays, the development of assays for intrahepatic covalently closed circular DNA (cccDNA) and replicative intermediates, the earlier detection of drug-resistant mutants, and quantitative serologic assays. Quantitative serologic assessment of virologic factors plays a pivotal role in the diagnosis and effective management of chronic hepatitis B (CHB) [1]. Highly sensitive assays for the quantification of HBV DNA have become a primary tool in selecting patients who are candidates for therapy, monitoring response to therapy, and detecting the emergence of drug resistance [2–5]. Highly sensitive polymerase chain reaction (PCR) based assays have become the standard in the clinical development of new antiviral therapies [6–10].

In addition to HBV DNA levels, several clinical investigations have provided evidence that relationships exist between other viral antigens, such as hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg), and the natural course of disease as well as patients' response to antiviral therapy [11–15]. Accordingly, the development of quantitative assays for key viral antigens, including HBeAg and HBsAg, has become a focus of the next phase of translational and clinical research. This article reviews the current literature regarding the quantitative assessment of

HBeAg and HBsAg in the context of understanding the natural history of CHB. Also discussed is the emerging role of HBeAg and HBsAg levels in guiding management decisions during antiviral therapy.

Quantitative HBsAg and HBeAg assays

As shown in Table 1, several diagnostic assays for the quantification of HBeAg and HBsAg have been developed [15–19]. Initial methods for the quantification of HBeAg and HBsAg used a variety of techniques, including radioimmunoassay, fluorescence, and chemiluminescence (enzyme-linked immunosorbent assay), and electro-immunodiffusion [12, 15, 18, 20]. A fully automated chemiluminescent microparticle immunoassay (Architect® HBsAg QT; Abbott Laboratories, Abbott Park, IL, USA) was first described by Deguchi et al. [16] for the detection and quantitation of HBsAg. In this study, the sensitivity of the Architect HBsAg QT assay was found to be approximately 0.2 ng/ml, which is equivalent or superior to other known and commercially available enzyme immunoassays and/or chemiluminescent immunoassays.

The Architect platform has become the most widely used assay for the quantification of HBeAg and HBsAg levels. It is also arguably the most convenient assay, possibly because it is fully automated and has a high throughput capacity. The Architect quantitative HBsAg assay is based on a calibration curve standardized to the World Health Organization criterion for HBsAg [16]. Quantitative HBsAg levels are reported in IU/ml, with a reactive range of 0.05–250.00 IU/ml. Given that HBsAg titers are often above this range, serum dilutions of 1:100–1:1000 are often required. This assay is also able to qualitatively detect surface gene mutants [21]. In the Architect platform, the HBeAg assay is designed to be semiquantitative and is reported as sample/cutoff (S/CO). However, it has a reasonable linear range and can be further modified and optimized for use as a quantitative assay by reference to an external standard, such as the Paul Ehrlich Institute (Langen, Germany) reference standard for HBeAg (results expressed in *Paul Ehrlich Institute*, PE IU/ml) [12, 22].

The availability of assays for HBeAg and HBsAg quantification has spurred investigation of the expression and role of these HBV serologic markers during the natural course of acute and chronic HBV infection. Findings from these studies indicate that the quantitative determination of HBsAg and HBeAg in addition to HBV DNA quantification may be useful in the diagnosis and follow-up of chronically infected patients [13, 16]. A brief discussion follows on the role of HBeAg and HBsAg in the natural history of CHB and predicting response to anti-HBV therapy.

Quantitative HBeAg

HBeAg is highly conserved evolutionarily among all hepadnaviruses. HBeAg is an accessory protein of HBV, and can be detected within the hepatocyte as well as being efficiently secreted. It is not required for viral replication but is thought to be essential for the development of chronic infection. Indeed, there are no reports of initial infections with HBeAg-negative variants progressing to chronic infection. Findings from preclinical studies conducted in transgenic mouse models of HBV-specific immune tolerance indicate that

Table 1 Reported assays for HBeAg and HBsAg quantitation

Type of assay	Method
Electro-immunodiffusion (QIE Laurell method) [15]	A combination of two techniques: immunoprecipitation and electrophoresis Serum samples are loaded onto an agarose gel containing relevant antibody (HBe or HBs). Precipitates formed in electrophoresis can then be quantified against a known standard
Immunoassays	
Sandwich radioimmunoassay [18]	HBeAg from serum can bind to human polyclonal anti-HBe-coated beads. Incubation with human polyclonal ^{125}I-anti-HBe can detect and quantify HBeAg
Architect platform (Abbott Laboratories, Abbott Park, IL, USA) [16]	A two-step chemiluminescent microparticle immunoassay that initially involves the combination of sample (serum/plasma) with either anti-HBs or anti-HBe coated paramagnetic microparticles. Following washing, the second step involves addition of acridinium-labeled antibody conjugate. The resulting chemiluminescent reaction is measured as relative light units
Elecsys HBsAg II (Roche Diagnostics, Indianapolis, IN, USA) [17, 19]	Sandwich complex formed by serum sample with two biotinylated monoclonal HBsAg-specific antibodies, a mixture of monoclonal HBsAg-specific antibody, and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex. Following the addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier

HBeAg may act as either a tolerogen or an immunogen, depending on the phase of chronic infection [23].

The measurement of HBeAg and natural history of CHB

The natural course of chronic HBV infection can be characterized by four distinct phases: an immune-tolerant phase, an immune-clearance phase, a low replicative phase, and an HBeAg-negative hepatitis disease phase [24]. The patient's serologic profile with respect to HBV DNA, HBsAg, and HBeAg levels changes with transition through the different phases of CHB. Substantial evidence has shown that suppression of HBV replication below the level of detection using a PCR-based assay is a critical factor in reducing the development of liver complications and liver disease progression in cirrhosis and hepatocellular carcinoma [1]. Thus, rapid and durable HBV suppression is the primary clinical goal for the treatment of patients with CHB [2–4].

HBeAg seroconversion is also considered an important factor in the natural history of HBeAg-positive patients with CHB. HBeAg, a circulating protein derived from translation of the core gene, appears first during acute HBV infection, and its detection is indicative of active virus replication, whereas loss of HBeAg and detection of antibody to HBeAg (anti-HBe) are associated with low to undetectable viral replication, remission of liver disease, and an increased likelihood of HBsAg seroconversion [24–27]. Current professional society and expert guidelines for the management of CHB consider HBeAg seroconversion, as well as suppression of serum HBV DNA with undetectable HBV DNA levels documented on two separate occasions at least 6 months apart, a critical therapeutic milestone and a potential stopping point for therapy in the management of HBeAg-positive CHB [2–4]. Therefore, while HBeAg seroconversion is a desirable end point for antiviral therapy, suppression of HBV DNA levels is critical in preventing and delaying the occurrence of liver complications.

Unfortunately, current commercial serologic assays for both HBeAg and anti-HBe are qualitative only and thus can diagnose HBeAg seroconversion only after the event has occurred. Using sensitive immunoassays, it has been recognized that anti-HBe can be detected in the presence of circulating serum HBeAg. In some cases, anti-HBe could be detected years prior to HBeAg seroconversion [28]. Thus, a humoral response with positive anti-HBe levels (in the presence of excess HBeAg) could be detected, noted not only during the immune-clearance phase but *also* in a significant proportion of patients traditionally classified as immune tolerant. Longitudinal analysis of quantitative HBeAg could confirm the hypothesis of high HBeAg titers in the immune-tolerant phase, with a gradual decline through transition into the immune-elimination phase, and eventual HBeAg seroconversion.

Patients in the immune-clearance or HBeAg-negative hepatitis disease phase are *the* potential treatment candidates and have been studied extensively in clinical trials. However, much less is known about patients in the immune-tolerant phase. Even less is known about patients with HBV infection during pregnancy, where HBeAg positivity and high viral DNA loads may be associated with vaccine failure in the neonate [29].

Issues, such as relative HBeAg titers in the different phases of CHB, and whether a critical HBeAg threshold exists in the immunopathogenesis of CHB, are currently unknown. Similarly, the precise relationship among HBeAg and cccDNA, HBV DNA, HBsAg, and serum alanine aminotransferase (ALT) is also unknown. Initial studies found no correlation between quantitative HBeAg and HBV DNA but were limited by older HBV DNA assays of lower sensitivity [11, 12]. Clearly, quantitative HBeAg serology, in conjunction with sensitive viral load assays, genotypic analysis, and innate/adaptive immune response markers, could improve the conceptual understanding of the natural history of CHB. This may lead to further classification of the immune-tolerant phase and possibly stratify patients into those likely to achieve an early, spontaneous HBeAg seroconversion from patients who would benefit from therapeutic intervention.

There are two important caveats to the interpretation of quantitative HBeAg: the effect of precore and basal core promoter mutations of HBeAg levels and phase of CHB. Mutations in the precore and basal core promoter regions of HBV arise under host immune pressure and may prevent the translation of HBeAg or reduced expression of HBeAg [30, 31]. Given that these mutations can exist as quasispecies in patients with HBeAg-positive disease and can be associated with both lower HBeAg titer and altered serum viral load in comparison with wild-type virus, stratification into dominant virus may be necessary to interpret an HBeAg level. The phase of CHB should also be taken into consideration when interpreting quantitative HBeAg. HBeAg levels generally are higher during the immune-tolerant phase and decrease with increasing age as the individual's adaptive immune response develops and anti-HBe titers increase [24]. Accurate classification of the phase of CHB relies on determination of serum ALT levels.

The measurement of HBeAg and response to therapy

Pegylated interferon therapy

In HBeAg-positive CHB, HBeAg seroconversion occurs in approximately 30% of patients treated with 48 weeks of

pegylated interferon alfa (peginterferon alfa) [32, 33]. HBeAg seroconversion rate has been shown to increase following cessation of therapy [32]. Pretreatment predictors of increased response to peginterferon alfa therapy in patients with HBeAg-positive CHB include high pretreatment ALT levels (>2× upper limit of normal), low viral load, increased histologic activity, young age, female gender, and genotypes A and B [17, 33–35]. The use of HBeAg as a potential biomarker in predicting responses to peginterferon alfa therapy and, thus, guiding management algorithms is beginning to emerge. Studies evaluating the dynamic changes in quantitative HBeAg titer have been conducted with both standard interferon alfa and peginterferon in adults as well as children [20, 22, 36–38]. In these studies, virologic responders tend to have either lower pretreatment HBeAg titers or display significant decline during therapy. Furthermore, HBeAg loss during the first 32 weeks of therapy has been suggested to be a predictor for HBsAg clearance at long-term follow-up [39]. Unfortunately, not all studies measured HBeAg against a reference standard; thus, it is difficult to directly compare studies. The effectiveness of quantitative HBeAg in predicting HBeAg seroconversion in patients treated with peginterferon alfa-2a was recently reported [22]. This analysis involved 271 HBV-infected HBeAg-positive patients who received peginterferon alfa-2a plus oral placebo for 48 weeks. HBeAg levels were measured serially during therapy using a microparticle enzyme immunoassay validated with in-house reference standards obtained from the Paul Ehrlich Institute (PE IU/ml). In patients who achieved HBeAg seroconversion, levels of HBeAg consistently decreased during treatment and remained at their lowest level during the 24 weeks of posttreatment follow-up. A critical pretreatment HBeAg level of ≤31 PE IU/ml was associated with an increased likelihood of HBeAg seroconversion (Table 2) [22]. Nonresponders had higher baseline titers, and HBeAg titer declined to a lesser degree with therapy, rebounding after treatment was discontinued. Furthermore, at week 24, the negative predictive value of an HBeAg titer of >100 PE IU/ml was greater than that of serum HBV DNA (96 vs. 86%) [22].

Table 2 Quantitative HBeAg as a tool to predict HBeAg seroconversion at 6 months after therapy with 48 weeks of peginterferon alfa-2a

	HBeAg seroconversion rate (%)
Baseline HBeAg (PE IU/ml)	
≤31	54[a]
31–1294	26
>1294	24
Week 24 HBeAg (PE IU/ml)	
≤10	52
10–100	20
>100	4 (96 NPV)

Fried et al. [22]

[a] $P < 0.001$

A "stopping rule" already exists for the peginterferon-based treatment of patients with chronic hepatitis C [40, 41]. Given the potential adverse effects of peginterferon therapy, similar algorithms would be of value in patients with CHB. This may eventually be possible with the use of quantitative HBeAg, alone or in combination with other potential markers such as HBV DNA and viral genotype. However, the published literature suggests different quantitative HBeAg time points as the optimal times to predict virologic response [12, 20, 22]. Further studies are required to better evaluate the dynamic changes and predictive power of HBeAg titers. This can really be facilitated only by a standardized and widely available commercial assay for quantitative HBeAg measurement, which is unfortunately currently lacking.

Oral nucleos(t)ide analog therapy

The advent of highly potent oral nucleos(t)ide analog (NA) therapies such as entecavir and tenofovir has resulted in the rapid suppression of viral replication, often to undetectable levels, using sensitive viral load assays. However, this increased potency does not translate into higher HBeAg seroconversion, with similar rates compared with older medications such as lamivudine. Approximately 20% of HBeAg-positive CHB patients achieve HBeAg seroconversion at week 48 of oral NA therapy [6, 7, 9]. Thus, a significant proportion of patients require prolonged treatment and are consequently at an increased risk of developing antiviral resistance.

Data on factors predictive of HBeAg loss or HBeAg seroconversion induced by oral NA therapy are sparse. Multivariate analysis of factors predictive of HBeAg loss in 541 patients treated with lamivudine monotherapy found that elevated baseline ALT levels ($P < 0.001$) and histologic activity index score ($P < 0.001$) were important predictors of HBeAg loss in response to lamivudine [42]. Given the limited utility of HBV DNA in predicting HBeAg seroconversion, quantitative HBeAg may play a role in predicting virologic response. It is important to consider that the mechanism of action of oral NA therapy is to directly inhibit HBV polymerase and viral replication. HBeAg synthesis is not directly affected. As shown in Fig. 1, the pathways of HBV DNA replication and HBeAg are separate. However, a reduction in viral replication results in decreased cccDNA "recycling" and consequent reduction in transcription of pregenomic RNA in the longer term [43]. Thus, HBeAg synthesis can be affected

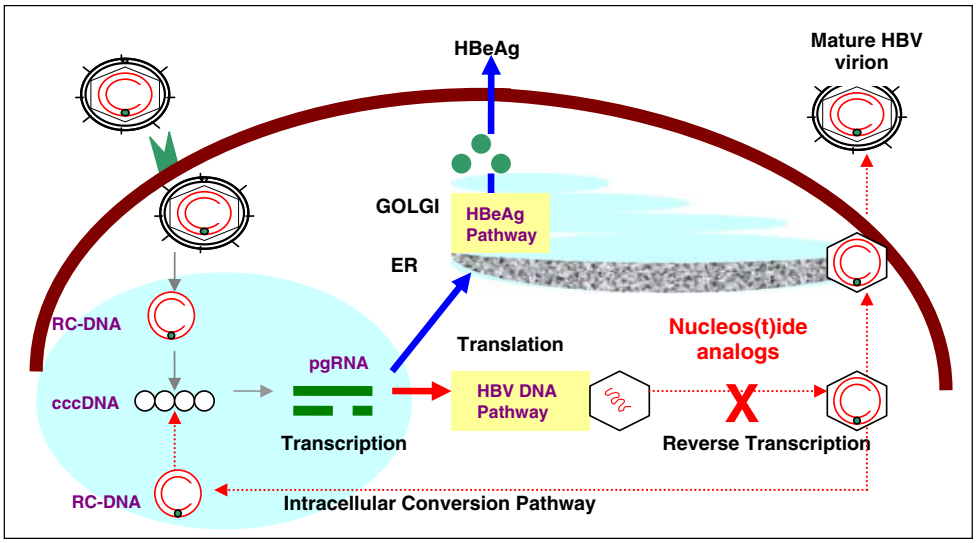

Fig. 1 The separate pathways of HBV polymerase and HBeAg. Oral NA therapy can indirectly affect HBeAg synthesis through a reduction in the intracellular conversion pathway

indirectly and would be expected to decline only after the initial phase of viral load suppression. Indeed, a lag phase between HBV viral load and HBeAg decline on potent antiviral therapy has been demonstrated recently [44].

To date, the published literature on quantitative HBeAg during NA therapy is predominantly retrospective [45, 46]. Shin et al. [45] retrospectively analyzed the predictive nature of quantitative HBeAg levels at different times of therapy in 220 HBeAg-positive patients with CHB who had received lamivudine for more than 24 months. A second study retrospectively analyzed the predictive value of HBeAg seroconversion and viral breakthrough during lamivudine therapy in 340 treatment-naive HBeAg-positive patients with CHB who were treated with lamivudine [46]. The mean duration of lamivudine therapy in this study was 18.7 months (range 6–56 months). In both studies, patients were classified based on dynamic quantitative HBeAg patterns in decrescendo, decrescendo-crescendo, no change, or fluctuating groups. In addition to serum ALT levels and duration of therapy, a decrescendo HBeAg pattern was also predictive of HBeAg seroconversion [45, 46]. Although HBeAg titers appeared to predict viral breakthrough earlier than did HBV DNA, less-sensitive assays were used (Digene® signal amplification; Digene Corporation, Gaithersburg, MD, USA; lower limit of detection, $\sim 2.8 \times 10^5$ copies/ml) [45].

A more recent, prospective study evaluated quantitative HBeAg with the emergence of lamivudine-resistant mutations in 76 HBeAg-positive naive CHB patients treated with lamivudine for a median of 52 weeks [47]. Viral load was measured using a COBAS® Amplicor HBV Monitor kit (Roche Molecular Systems, Pleasanton, CA, USA; lower limit of detection, 200 copies/ml). Quantitative HBeAg was recorded as a semiquantitative S/CO value. HBeAg breakthrough was defined as a >50 S/CO increase in HBeAg titer above nadir after the achievement of a progressive 90% reduction [47]. In this study, although quantitative HBeAg was useful in predicting the emergence of lamivudine-resistant mutants, viral breakthrough was detected a median of 4 weeks earlier. Thus, although quantitative HBeAg may play a role in predicting HBeAg seroconversion in patients receiving oral NA therapy, it currently does not appear to be more beneficial than serum viral load in detecting the emergence of antiviral resistance.

Following HBeAg seroconversion, the durability of the virologic response becomes the next critical clinical question. Factors associated with a more durable HBeAg seroconversion following lamivudine therapy include longer duration of consolidation treatment, younger age, lower end of treatment viral load, and genotype B compared with genotype C [5]. The durability of HBeAg seroconversion with NA therapy appears inferior to that of immunomodulator therapy. Investigation of dynamic on- and off-treatment quantitative HBeAg serology may allow better prediction of HBeAg seroconversion and permit refinement of consolidation treatment protocols to improve durability.

Quantitative HBsAg

The hallmark of CHB infection is the presence of HBsAg positivity for at least 6 months. HBsAg is encoded by the HBV envelope gene, which contains three open-reading frame "start" codons: the pre-S1, pre-S2, and ORF-S domains. Transcription and subsequent translation result in small (ORF-S), medium (pre-S2 + ORF-S), and large (pre-S1 + pre-S2 + ORF-S) HBsAg proteins. Newly synthesized HBsAg proteins are incorporated into the

mature HBV nucleocapsids at the endoplasmic reticulum prior to secretion from the hepatocyte. However, HBsAg production far exceeds that required for virion assembly, and remaining HBsAg can also be secreted as noninfectious filamentous or spheric subviral particles. Similar to the HBeAg pathway, HBsAg synthesis is separate from the viral replication pathway. Thus, oral NA therapy also does not directly affect HBsAg production. Quantitative HBsAg serology can detect all three forms of HBsAg in the circulation but cannot differentiate their relative proportions. The relative forms of HBsAg in different phases of CHB are currently unknown.

The measurement of HBsAg and natural history of CHB

Covalently closed circular DNA

Intrahepatic cccDNA levels represent the infected liver cell burden. Interest in quantitative HBsAg serology has been founded on initial studies demonstrating a positive association with intrahepatic cccDNA levels [13, 16]. Studies assessing cccDNA levels during the natural history of CHB have consistently demonstrated that cccDNA levels are higher in HBeAg-positive patients [15, 43, 48, 49]. However, preliminary evidence from recent studies has demonstrated conflicting associations [50, 51]. The most common method for measuring cccDNA levels is invasive and requires liver biopsy specimens [43]. In contrast, quantitative HBsAg testing requires only a serum sample, and the Architect QT assay is fully automated and standardized. Thus, quantitative HBsAg serology being performed on a reliable testing platform is an attractive research tool with potential clinical utility. Quantitative HBsAg serology should be incorporated into the design of clinical trials, but further prospective studies are still required to define the exact relationship of HBsAg to cccDNA and in the context of the natural history of CHB.

HBV DNA

In clinical practice, HBV viral load determination by sensitive assays is essential in evaluating the natural history of CHB. Although a viral load of 10^5 copies/ml ($\sim 2 \times 10^4$ IU/ml) is used to differentiate patients in the non/low-replicative and HBeAg-negative phases [52], this cut-off threshold is not absolute. Quantitative HBsAg may play a future role in more clearly defining these phases.

Cross-sectional studies have positively correlated HBsAg titers with serum HBV DNA in both HBeAg-positive and -negative cohorts [16, 53, 54]. In parallel with viral load, HBsAg titers are generally also higher in HBeAg-positive patients. In one study of 67 patients, the study participants were stratified into one HBeAg-positive (viral load $>2 \times 10^7$ copies/ml) and three HBeAg-negative groups based on viral replication ($<10^3$, 10^{3-5}, and $>10^5$ copies/ml, respectively) [53]. A group of HBeAg-positive patients with lower viral loads ($<10^7$ copies/ml) was not included. A cut-off HBsAg level of 15,000 IU/ml was found to differentiate the HBeAg-positive group (viral load $>2 \times 10^7$ copies/ml) with 100% sensitivity and specificity. However, a proportion of HBeAg-negative patients with low HBsAg titers had persistently high levels of viral replication, suggesting a possible "disconnect" between viral replication and HBsAg synthesis. Although no quantitative HBsAg cut-off level could distinguish viral load cohorts of $<10^3$ and 10^{3-5} copies/ml, HBsAg levels were significantly lower between the $<10^3$ and $>10^5$ copies/ml groups.

Given that viral load assays are more expensive than quantitative HBsAg, a critical question is whether HBsAg can be used instead of, or must be used in conjunction with, HBV DNA levels. A recent study in Taiwan suggested that cut-off HBsAg titers of 314 and 768 IU/ml could predict serum HBV DNA levels of 2000 and 20,000 IU/ml, respectively ($\sim$80% sensitivity and 50–60% specificity) [54]. However, a wide range of pretreatment HBsAg levels have previously been observed in the immune-clearance phase of CHB [55]. Clearly, this observation needs to be confirmed. Thus, larger prospective and longitudinal studies are required to further investigate the significance of HBsAg in the immunopathogenesis and natural history of CHB.

The measurement of HBsAg and response to therapy

Peginterferon

HBsAg is cleared during recovery with acute infection, and an anti-HBs immune response following vaccination is generally protective against possible subsequent infection. In CHB, HBsAg seroconversion is considered the preferred end point because it is believed to represent successful immunologic control of HBV. Multiple studies have demonstrated that HBsAg seroconversion is associated with a favorable prognosis [56–58]. Spontaneous HBsAg loss is rare, however, with a 1–2% annual rate. Although HBsAg seroconversion is the closest end point to a "clinical cure," it is important to note that intrahepatic cccDNA can still be detected at low levels and, as such, represents a reservoir for potential disease reactivation [59].

Although HBsAg loss and seroconversion can be increased with a finite course of standard interferon alfa or peginterferon therapy, only a small proportion (3%) of

patients experience HBsAg loss following 48 weeks of therapy [60]. Furthermore, in long-term virologic responders to interferon, HBsAg loss can still occur while the patient is off therapy; thus, rates do steadily increase over time [61]. It is becoming increasingly clear that the potent suppression of viral replication alone is insufficient for HBsAg loss, and that it is the immune-modulating effect of interferon that results in higher HBsAg loss rates. Further study of the innate/adaptive immune system both during and off treatment is required to fully understand the mechanisms underlying HBsAg seroconversion.

The kinetics of HBsAg decline during immunomodulator therapy has been evaluated in patients receiving immunomodulator therapy alone or in combination with oral NAs [62, 63]. Baseline HBsAg levels appear to be genotype dependent (highest in genotypes A and D), and the overall HBsAg titer decline was also shown to be genotype dependent (highest in genotype B, lowest in genotype D) [62]. On-treatment HBsAg titers also appear to be associated with cccDNA levels [50, 55].

The role of quantitative HBsAg in predicting response to peginterferon therapy has been the focus of several recent studies that have been published in abstract form (Table 3) [50, 55, 62, 64–69]. Findings from these studies suggest that, in treatment-naive patients, the baseline HBsAg titer and/or on-treatment decline may help predict response to therapy and eventual HBsAg loss/seroconversion. In addition, the prediction of HBsAg loss at 4 years after treatment may be possible to identify as early as week 12 of treatment (Tables 4 and 5) [65, 70]. In patients with previous lamivudine resistance, peginterferon results in a greater decline in quantitative HBsAg, HBeAg seroconversion, and HBsAg loss than does adefovir [64].

Oral NA therapy

Oral NA therapy inhibits viral DNA replication in a biphasic pattern [71]. The first phase is rapid and is due to the direct inhibition of viral replication in infected hepatocytes. The second phase is slower and has been attributed to the clearance of infected cells containing cccDNA. Following 48 weeks of potent NA therapy, up to a 7-log reduction in HBV DNA levels can be achieved in HBeAg-positive patients [6, 8, 9]. In contrast, the rate of on-treatment cccDNA and HBsAg titer decline is lower (up to 1 log) [15, 43, 72].

Little data exist on the predictive value of quantitative HBsAg in the setting of oral NA therapy. In patients treated with lamivudine, pretreatment HBsAg titers have been correlated with sustained virologic response [73] and HBsAg seroconversion [51, 74]. Rising HBsAg concentrations are associated with the emergence of lamivudine resistance [72]. A further potential application of quantitative HBsAg is in the HBeAg-negative setting, where a

Table 3 Recent studies evaluating quantitative HBsAg during immunomodulator therapy

Author	*N*	Treatment	HBeAg	Findings and potential role
Manesis et al. [66]	63	Interferon alfa-2b	−	Baseline HBsAg may predict HBsAg seroconversion
		LMV monotherapy		HBsAg decline on-treatment is greater for interferon alfa-2b than for LMV
Chan et al. [55]	26	Peginterferon alfa-2b + LMV	+	Baseline HBsAg may predict sustained virologic response
Takkenberg et al. [69]	70	Peginterferon alfa-2a + ADV	±	Baseline HBsAg titer predicts HBsAg clearance or seroconversion
				HBsAg clearance is higher with combination peginterferon alfa-2a + ADV than with monotherapy
Lu et al. [50]	86	Peginterferon alfa-2a ± LMV	±	Baseline HBsAg titer is superior to cccDNA and serum HBV DNA in predicting sustained virologic response
Lau et al. [65]	539	Peginterferon alfa-2a	+	HBsAg decline on treatment may predict HBeAg seroconversion at 1 year after treatment, as well as a durable off-treatment response
		Peginterferon alfa-2a + LMV		
Moucari et al. [67]	160	Peginterferon alfa-2a ± LMV	−	HBsAg level <1500 IU/ml at week 12 is associated with HBsAg clearance at 4 years after treatment (PPV, 35%). 97% of patients do not clear HBsAg if HBsAg is >1500 IU/ml at week 12
Rijckborst et al. [68]	133	Peginterferon alfa-2a	−	HBsAg decline at week 12 may predict sustained virologic response (HBV DNA <10,000 copies/ml 24 week after treatment)
		Peginterferon alfa-2a + RBV		
Brunetto et al. [62]	80	Peginterferon alfa-2a ± LMV	−	Extent of HBsAg decline on treatment may be genotype dependent
Hou et al. [64]	251	Peginterferon alfa-2a versus ADV in LMV-resistant patients	+	Decline of HBsAg, HBeAg seroconversion, and HBsAg clearance is greater with peginterferon than with ADV
				Peginterferon alfa-2a is an option in patients with previous LMV resistance

Table 4 Quantitative HBsAg as a tool to predict HBsAg loss and virological response at 6 months post-therapy in HBeAg-positive patients

	Week 12 HBsAg on Peginterferon alfa-2a ± LMV[a] (IU/ml)	HBV DNA ≤10000 copies/ml (%)	HBV DNA ≤400 copies/ml (%)	HBsAg loss (%)
HBeAg-positive	≤1500	46.8	31.2	10.1
	1501–20,000	22.6	11.1	1.8
	>20,000	8.2	4.1	3.3

Lau et al. [65]

[a] The percentage of patients with HBsAg titers of <1500, 1501–20,000, and >20,000 IU/ml at week 12 were 21, 55, and 24%, respectively

Table 5 Quantitative HBsAg as a tool to predict HBsAg loss and virologic response at 6 months and 4 years post-therapy in HBeAg-negative patients

	Week 12 HBsAg on Peginterferon alfa-2a ± LMV (IU/ml)	HBV DNA ≤10000 copies/ml		HBV DNA ≤400 copies/ml		HBsAg loss	
		6 months (%)	4 years (%)	6 months (%)	4 years (%)	6 months (%)	4 years (%)
HBeAg-negative	≤1500	59	39	39	31	7	23
	>1500	34	12	9	8	2	4

Marcellin et al. [70]

critical threshold HBsAg titer (a possible noninvasive surrogate for low cccDNA) may guide the appropriate duration of therapy and allow a trial of therapy cessation.

Conclusion

In the past decade, there has been an explosion of knowledge and research in the field of hepatitis B. This has been facilitated by the advent of improved diagnostic assays and, in particular, molecular techniques. In the future, baseline HBsAg quantitation may help refine the treatment algorithms for peginterferon in both treatment-naive and NA-resistant cohorts. Dynamic on-therapy HBsAg quantitation may improve the prediction of virologic response to peginterferon therapy as well as in the monitoring of treatment response to both peginterferon and oral NA therapy. HBsAg quantitation may also have future clinical utility in determining the optimal dose and duration of peginterferon therapy (e.g., the role of induction dosing), as well as evaluating whether combination or sequential therapy (peginterferon plus NA) confers any additional long-term benefit. Future studies may also evaluate whether the achievement of serum HBsAg levels similar to those typically seen in the low replicative phase could be a therapeutic end point on the way to potential HBsAg seroclearance.

Although the application of quantitative HBeAg and HBsAg promises to be a valuable clinical tool in the near future, large prospective studies using standardized assays for HBeAg and HBsAg, evaluating longitudinal changes in quantitative HBeAg and HBsAg, are required to validate the current published literature.

It is envisaged that the future understanding of the natural history of CHB will continue to evolve and that management algorithms will become more individualized, incorporating not only quantitative serology but, possibly, also genotyping and markers of the innate and adaptive immune responses.

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