Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Hepatitis B surface antigen quantification: Why and how to use it in 2011 – A core group report

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Quantitative HBsAg had been suggested to be helpful in management of HBV, but assays were cumbersome. The recent availability of commercial quantitative assays has restarted the interest in quantitative serum hepatitis B surface antigen (HBsAg) as a biomarker for prognosis and treatment response in chronic hepatitis B. HBsAg level reflects the transcriptional activity of cccDNA rather than the absolute amount of cccDNA copies. Serum HBsAg level tends to be higher in hepatitis B e antigen (HBeAg)-positive than HBeAg-negative patients. Among patients with a low HBV DNA (<2000 IU/ml), HBsAg <1000 IU/ml in genotype D HBV infection and HBsAg <100 IU/ml in genotype B/C HBV infection is associated with inactive carrier state in HBeAg-negative patients. The HBsAg reduction by nucleos(t)ide analogues (NA) is not as pronounced as by interferon treatment. On peginterferon treatment, sustained responders tend to show greater HBsAg decline than the non-responders. The optimal on-treatment HBsAg cutoff to predict response needs further evaluation in HBeAg-positive patients, but an absence of HBsAg decline together with a <2 log reduction in HBV DNA at week 12 can serve as stopping rule in HBeAg-negative patients with genotype D HBV infection. A rapid serum HBsAg decline during NA therapy may identify patients who will clear HBsAg in the long-term. There are early reports among Asian patients that an HBsAg level of <100 IU/ml might predict lower risk of relapse after stopping NA treatment. In clinical practice, serum HBsAg level should be used together with, but not as a substitute for, HBV DNA.

Keywords: Hepatitis B virus; HBsAg, Antiviral treatment; Peginterferon.
Received 26 March 2011; received in revised form 27 May 2011; accepted 9 June 2011
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Abbreviations: Anti-HBe, antibodies to hepatitis B e antigen; ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; NA, nuclet(s)ide analogues; NPV, negative predictive value; ORF, open reading frame; PPV, positive predictive value.

Introduction

Since the discovery of hepatitis B surface antigen (HBsAg) by Blumberg in 1965, it has been used as the hallmark for the diagnosis of hepatitis B virus (HBV) infection [1]. The importance of HBsAg quantification was recognized early on, but initial tests were labor intensive and thus restricted to the research setting [2]. In addition, no treatment for HBV was available in the 1980s when those labor-intensive tests were developed and, therefore, these tests did not become clinically relevant. Although standard assays have been available for quantification of serum HBsAg for a number of years, it is the presence or absence of HBsAg that carries a clinical meaning to most physicians [3,4]. The interest in HBsAg quantification started with the possible observation of its association with the level of covalently closed circular (ccc) DNA, the template for viral replication inside the nuclei of hepatocytes [5,6].

In recent years, many studies have been performed, addressing the use of serum HBsAg level to monitor the natural history and predict treatment response in chronic hepatitis B (CHB). In light of the availability of the new data, a group of hepatologists met in September 2010 (in Asian Pacific Digestive Week, Kuala Lumpur, Malaysia), October 2010 (in the Annual meeting of American Association for the Study of the Liver, Boston, United States) and February 2011 (in the Annual meeting of Asian Pacific Association for the Study of the Liver, Bangkok, Thailand) with the unrestricted support of F. Hoffmann-la Roche to discuss the role of serum HBsAg quantification in clinical practice. An independent literature

search was performed by the group members. Data were derived from the electronic databases and the abstracts of major international conferences. The review was based primarily on published full manuscripts and supplemented by new data from appropriate conference abstracts. The suggestions were based on solid evidence where possible, but on their own clinical experience and expert opinion where data were lacking. The industrial partner did not advise nor interfere with the content of this paper. The authors were responsible for writing the entire manuscript without the assistance of a medical writer. The manuscript is not meant to be a guideline or a position paper. The aim of this paper was to give insight to clinicians concerning the use of serum HBsAg quantification in clinical practice and to highlight the questions that need to be addressed by future studies.

Virology of hepatitis B surface antigen

Current evidence

HBsAg is the glycosylated envelope protein of the mature HBV virion. There are three HBsAg proteins – small (S), medium (M), and large (L). The envelope open reading frame (ORF) contains three in frame "start" codons which further divide it into pre-S1, pre-S2, and S-ORF domains (Fig. 1). There are two HBV mRNA transcripts, Pre-S1 mRNA, and Pre-S2/S mRNA, from which the L (Pre-S1) and M/S (Pre-S2 + S/S) proteins are translated, respectively. HBV replication occurs via the pregenomic RNA (pgRNA), a separate RNA transcript, and, therefore, the HBsAg secretory pathway and the viral replication pathway can be considered as distinct processes within the hepatocyte (Fig. 2).

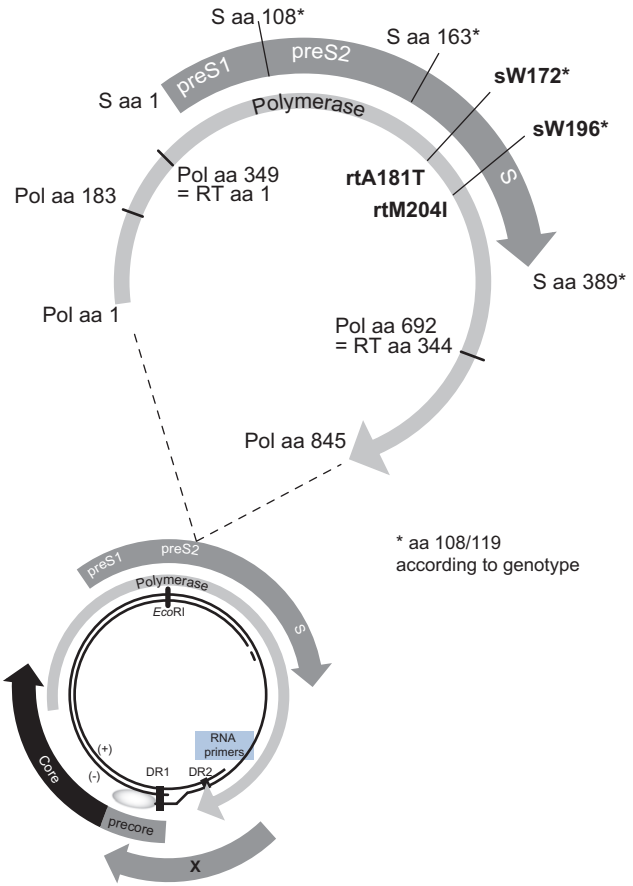

Fig. 1. The HBV open reading frames (ORF), highlighting the overlapping relationship between the envelope ORF and the HBV polymerase ORF.

As well as virions, the sera of viremic patients contain large numbers of two types of non-infectious particles: spherical particles and filamentous forms (Fig. 2). Both types of subviral particles are composed of HBsAg. The subviral particles are secreted at levels far in excess of mature virions, and are believed to play a role as a decoy for humoral immunity. HBsAg may also be produced from HBV DNA integrated into the host genome (Fig. 2). Although viral integration is not required for normal productive hepadnaviral infection, integration of HBV DNA occurs illegitimately through recombination mechanisms using host enzymes acting on the double-stranded linear DNA form of HBV [7,8]. In HBV infection, viral integration seems to occur early in infection. Integrated sequences cannot provide a template for productive viral replication as a complete genome is not present. However, given that sequences of the S-ORF with enhancer I elements are often present in integrated segments, HBsAg may be produced, often as truncated subviral particles [9].

HBV is unique among DNA viruses for replicating its genome via an RNA intermediate. The transcriptional template of HBV is the cccDNA which exists in the cell nucleus as a viral minichromosome and serves as the intrahepatic reservoir for HBV (Fig. 2). The challenge of antiviral therapy for CHB is to clear the liver of cccDNA. HBsAg titer should theoretically reflect liver total cccDNA, as well as the transcriptional activity of the cccDNA (Fig. 2). Indeed, it has been shown that HBsAg titers can correlate with serum HBV DNA and intrahepatic cccDNA levels, but that this may vary in the different phases of disease [10–12]. A positive correlation has been noted between HBsAg titer and serum HBV DNA and liver cccDNA in most studies of HBeAg-positive patients. However, the regulation of HBsAg production and secretion appears to be disconnected from that of virion production in the HBeAg-negative state [12,13]. In HBeAg-negative patients, although serum HBsAg titer, serum HBV DNA level and liver cccDNA level are all reduced relative to patients with HBeAg-positive CHB, HBsAg titers have been noted to be preserved relative to serum HBV DNA and cccDNA [12–14]. The reason for this observation is unclear, but may be related to the presence of integrated HBV surface genome into the host chromosome and/or a preferential control of the replicative pathway over HBsAg transcription/secretion, where virion production is inhibited but secretion of subviral particles is preserved [12,14]. Additional studies are needed to determine the utility of HBsAg measurement in HBeAg-negative patients.

HBV has the added complexity of overlapping reading frames. The envelope ORF completely overlaps the polymerase gene (Fig. 2). Therefore point mutations in the polymerase ORF that are selected by nucleos(t)ide analogue (NA) therapy may result in critical changes, including stop codons, in the envelope proteins. For example, the mutations rtA181T and rtM204I, associated with NA resistance, can result in the stop codons sW172* and W196* in the overlapping S-ORF, respectively [15]. It remains unclear whether these mutations, which arise as one population in a quasi-species, affect HBsAg titers. They have been associated with hepatocyte HBsAg retention and potential oncogenic risk [16].

Finally, from a diagnostic perspective, it is important to appreciate that HBsAg quantification detects all three forms of circulating HBsAg. The antibodies used in the quantitative

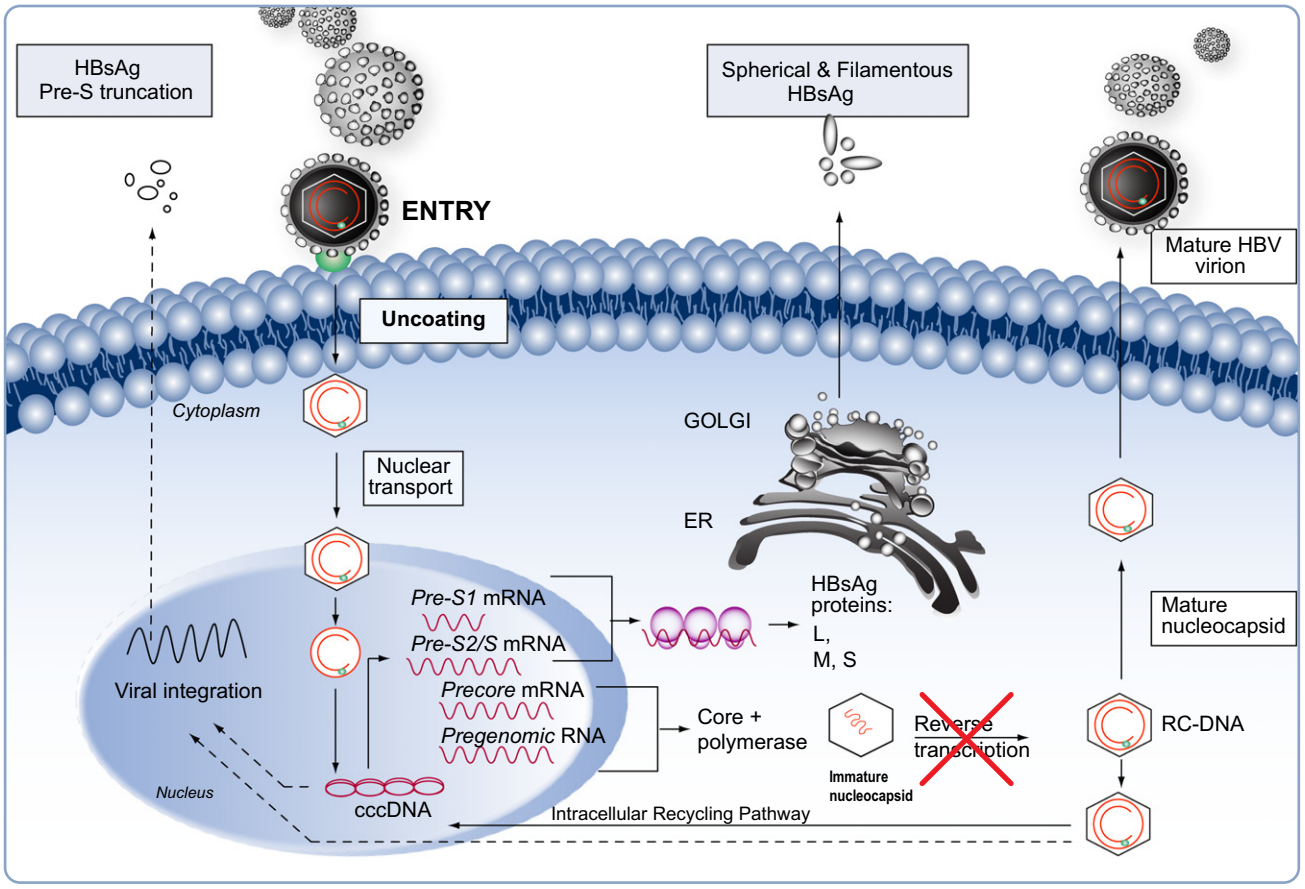

Fig. 2. HVB lifecycle. The lifecycle of HBV, highlighting: (i) the nuclear reservoir, covalently closed circular (ccc) DNA, which is the transcriptional template for the virus; (ii) the HBsAg secretory pathways, and (iii) the viral replication pathways. Nucleos(t)ide analogue therapy, by targeting the HBV reverse transcriptase (RT), selectively inhibits virion production, but does not reduce HBsAg levels as cccDNA levels are preserved. IFN-based strategies, which can non-cytolytically clear hepatocytes of HBV cccDNA infection, may therefore induce larger reductions in HBsAg.

enzyme immunoassays target epitopes in the S protein, and are therefore not capable of distinguishing between the different HBsAg proteins, nor can they distinguish between virion-associated HBsAg, subviral particles and HBsAg produced from integrated sequence. Currently, there are two commercialized assay that can measure the HBsAg quantification, the Architect QT assay (Abbott Laboratories) and the Elecsys HBsAg II Quant assay (Roche Diagnostic). The Architect assay can measure HBsAg level from 0.05 to 250 IU/ml, and manual dilution is needed for measurement of higher HBsAg levels. The Elecsys II assay has an automatic on-board dilution with a range of HBsAg measurement from 0.05 to 52,000 IU/ml. In a study among 611 chronic hepatitis B patients from 4 sites covering HBV genotype A–G, approximately 72% of samples could have HBsAg level measured on the first analysis by the Elecsys II assay without the need of further manual dilution [17]. There is good correlation between the HBsAg measurements by these 2 assays [18,19].

Natural history

HBeAg-positive chronic hepatitis B

Current evidence

In perinatally acquired HBV infection from infected mothers with positive hepatitis B e antigen (HBeAg), immune tolerance will be induced by the HBeAg that crosses the placenta. Immune tolerance phase usually occurs in the initial 2–3 decades of life characterized by positive HBeAg, very high HBV DNA, normal alanine aminotransferase (ALT) levels, and minimal histologic damage [20]. It is followed by an immune clearance phase, which may lead to HBeAg seroconversion. However, some patients have prolonged but unsuccessful immune clearance with persistently elevated HBV DNA and ALT levels, and these patients have a high risk of developing liver cirrhosis.

Based on the results of two cross-sectional studies, the serum HBsAg level was generally higher among patients in the immune tolerance phase than in the immune clearance phase [10,11]. In the European study comparing 30 patients in the immune tolerance phase versus 48 patients in the immune clearance phase, the mean serum HBsAg level was 4.96 log IU/ml *vs.* 4.37 log IU/ml, respectively [11]. In the Asian study, the mean serum HBsAg level was 4.53 log IU/ml among patients in the immune tolerance phase (n = 32) *vs.* 4.03 log IU/ml among patients in the immune clearance phase (n = 55) [10]. One possible reason for the lower serum HBsAg levels among patients in the immune tolerance phase in the Asian study was the inclusion of patients with ALT at 1–2 times upper limit of laboratory normal, which might actually represent early immune clearance. Another explanation may be the difference in HBsAg expression related to different HBV genotypes. In an experiment using HBV infected cell lines, HBsAg

expression was highest among HBV subgenotype A2/Ae, followed by A1/Aa and B2/Ba, and distantly by B1/Bj as well as C, and the least for D [21].

In a longitudinal study including untreated CHB patients followed up for 99 ± 16 months, serial HBsAg levels among chronic hepatitis B patients at different stages of disease were compared [22]. HBsAg level was persistently high at approximately 5 log IU/ml among patients in the immune tolerance phase, and HBsAg level tended to be very stable with a median annual decline of −0.006 log IU/ml. The HBsAg level among patients in the immune clearance phase was approximately 4 log IU/ml, and no difference could be detected between patients who had persistently active disease and those who underwent HBeAg seroconversion. The HBsAg levels also tended to be very steady among HBeAg-positive patients with active disease with a median annual decline of 0.021 log IU/ml.

The ratio of HBsAg to HBV DNA, which reflects the proportion of subviral particles to virions was very similar among all HBeAg-positive patients. In various studies, the median HBsAg to HBV DNA ratio ranged between 0.5 and 0.6 in all HBeAg-positive patients, with individual patients ranging between 0.1 and 2.0 [10,11,22].

Suggestions

Serum HBsAg level is higher in the immune tolerance phase than in the immune clearance phase. A very high HBsAg level of approximately 100,000 IU/ml can be a supportive evidence of immune tolerance. Hence HBsAg can provide additional information to differentiate immune tolerance and immune clearance when the HBV DNA is high and ALT is normal or minimally elevated. HBsAg level or HBsAg/HBV DNA ratio should not be used to predict the chance of spontaneous HBeAg seroconversion.

Challenges

More research is required to explore the rate of HBsAg reduction on the possibility of successful immune clearance in HBeAg-positive patients. There is preliminary data suggesting lower HBsAg level is associated with more severe liver fibrosis in HBeAg-positive patients [23]. This is an important area as HBV DNA level has no association with the severity of liver fibrosis in HBeAg-positive patients [24].

HBeAg-negative chronic hepatitis B

Current evidence

Later in life, most of the HBV infected population evolves into the immune clearance phase, losing HBeAg and seroconverting to antibodies to HBeAg (anti-HBe). Because of the aging of the infected population, the prevalence of HBeAg-negative CHB seems to be increasing worldwide [25–27]. HBeAg-negative CHB is characterized by wide fluctuation of serum HBV DNA levels and biochemical activity [28]. In patients with HBeAg-negative CHB, viremia may fall below 2000 IU/ml (the threshold identifying inactive HBV infection) even if transiently, thus for the differential diagnosis between HBeAg negative chronic hepatitis B and "inactive HBV carrier", serial determinations of serum ALT and HBV DNA levels are needed [29,30]. The inactive carriers have no or mild liver histological lesions, with an excellent survival prognosis and low incidence of cirrhosis and HCC, while patients with HBeAg-negative CHB with fluctuating activity have more severe progression of the disease with frequent development of cirrhosis [30–33]. According to the American and European guidelines [34,35], the differentiation between the inactive and active phases of HBeAg-negative CHB is based on an HBV DNA cut-off set up at 2000 IU/ml. This cut-off has led to several controversial reports [30,36–38].

Based on several longitudinal studies, HBsAg levels are higher in patients with active HBeAg-negative CHB than in "inactive carriers". A longitudinal study in Hong Kong reported, on 68 HBeAg-negative CHB patients predominantly infected with genotypes B and C HBV and followed for over 8 years, that the patients with an inactive disease tended to have lower HBsAg levels than those who had active disease; 2.24 ± 1.61 log IU/ml *vs.* 2.98 ± 0.88 log IU/ml, respectively ($p = 0.054$) [22]. In an extended report among 103 HBeAg-negative CHB patients followed up for a median of 11 years, serum HBsAg of ⩽100 IU/ml had 75% sensitivity and 91% specificity to predict spontaneous HBsAg seroclearance [39].

In Taiwan where genotype B and C HBV are prevalent, a cross-sectional study on 251 chronic hepatitis B patients showed that mean serum HBsAg level was higher in the immune clearance phases (3.81 log IU/ml), significantly decreased in the inactive carrier state (2.25 log IU/ml) and re-elevated in the reactivation phase (2.77 log IU/ml) [40]. In another report including 390 patients who experienced spontaneous HBeAg seroconversion, serum HBsAg level at 1 year post HBeAg seroconversion was inversely associated with the probability of HBsAg loss in a dose–response manner. Compared to patients with HBsAg levels ⩾1000 IU/ml, the rate of HBsAg loss was higher for those with HBsAg levels of 100–999 and <100 IU/ml, with hazard ratios of 4.4 (95% confidence interval, 1.1–17.0) and 24.3 (8.7–67.5), respectively. In patients with serum HBV DNA levels <200 IU/ml, serum HBsAg level <100 IU/ml predicted HBsAg loss within 6 years with a positive predictive value (PPV) of 45.5% and negative predictive value (NPV) of 98.6% [41].

A study performed in Italy investigated serum HBsAg levels and their diagnostic role in genotype D HBeAg-negative HBV carriers: 209 carriers where prospectively followed-up for a median period of 34.5 (6–110) months and classified as having active or inactive infection according to the virologic profile during 1 year of monthly monitoring [42]. Serum HBsAg levels were significantly lower in inactive (56 carriers) than in active infection (153 carriers): median values 62.12 (0.1–4068) IU/ml *vs.* 3029 (0.5–82,480) IU/ml, respectively (p <0.001). Among inactive carriers, HBsAg serum levels were lower in 31 asymptomatic carriers whose viremia remained persistently below 20,000 IU/ml than in 122 asymptomatic carriers with fluctuations >20,000 IU/ml: 883 (0.5–7838) *vs.* 4233 (164–82,480) IU/ml ($p = 0.002$). The combination of a single measurement of HBsAg <1000 IU/ml and HBV DNA <2000 IU/ml allowed identifying "inactive carriers" with a PPV 87.9% and NPV 96.7%.

Similar results were observed in a French study performed in 122 HBeAg-negative CHB (genotypes A–E) [43]. One hundred and two were inactive carriers (three normal ALT measurements in 1 year) and 20 were patients with HBeAg-negative CHB. HBsAg levels were lower in the 102 inactive carriers than in the 20 patients with HBeAg-negative CHB; 3.30 ± 0.97 *vs.* 3.77 ± 0.11 log IU/ml, respectively (p <0.001). At baseline, 50 of 54 (92.5%) patients who had HBsAg ⩽2000 IU/ml and 53 of 57 (93%) patients who had HBV DNA ⩽2000 IU/ml were inactive carriers. Overall, 32 patients had HBsAg ⩽2000 IU/ml and HBV DNA ⩽2000 IU/ml, and all were "inactive carriers" (PPV 100%). Similarly, one recent

Turkish study found that an HBsAg level of 2040 IU/ml best defined inactive carriers from chronic hepatitis patients during a 3-year follow-up (sensitivity 87.2%, specificity 75.3%) [44].

Suggestions

The combination of HBsAg and HBV DNA measurements at a single time-point might allow an accurate identification of "true inactive carriers" and prediction of HBsAg loss in the HBeAg-negative CHB population with distinct HBV genotype infection. It seems that HBsAg <1000 IU/ml is necessary but may not be sufficient to identify inactive carriers with HBV genotype D infection, and HBsAg <100 IU/ml can predict HBsAg loss over time in genotype B or C HBV-infected patients, respectively.

Challenges

The utility of this new marker needs further validation, for the management of HBeAg-negative CHB patients, in order to better identify those that could benefit from therapy and increased survival. Better identification of "true inactive carriers" or patients with a high likelihood of HBsAg loss, who have a good survival prognosis and need less frequent monitoring, will reduce the costs of medical expenses. However, HBsAg may also decrease with age and disease progression, and other clinical parameters including HBV DNA level must be taken into consideration to define inactive carrier state [45].

Peginterferon treatment

HBeAg-positive patients

Current evidence

Serum HBsAg levels show some correlation with intrahepatic cccDNA levels in patients with HBeAg-positive chronic hepatitis B, and decline in HBsAg may represent decline in cccDNA [12,22,46]. The degree of decline in HBsAg level during peginterferon treatment may reflect the efficacy of peginterferon and may provide useful information in prediction of treatment response. In an early study by Janssen *et al.*, serum HBsAg level has been shown to decrease dramatically among HBeAg-positive patients who responded to interferon therapy [47]. HBsAg level at the end of peginterferon treatment has also been shown to be significantly lower in patients with sustained response (defined as HBeAg seroconversion and HBV DNA <2000 IU/ml) 5 years post-treatment [48].

Current data indicate that lower baseline serum HBsAg levels are present in patients achieving sustained response post-peginterferon treatment compared to non-responders [49,50]. Early serological response, defined as low HBsAg level or greater HBsAg decline early during treatment, has been associated with higher HBeAg seroconversion and HBV DNA suppression 6 months post-treatment [51]. Chan *et al.* reported that sustained response (defined as HBeAg seroconversion and HBV DNA $\leqslant$2000 IU/ml at the end of peginterferon and/or lamivudine treatment, and that was sustained at 12 months post-treatment) was higher in patients who had HBsAg $\leqslant$300 IU/ml at month 6 during treatment (62% *vs.* 11% in those who had HBsAg >300 IU/ml at month 6, p <0.001) [49]. Patients having both HBsAg decline by greater than 1 log and serum HBsAg level $\leqslant$300 IU/ml at month 6 during treatment achieved a sustained response rate of 75% compared with 15% in those who did not have this combined response (p <0.001). The PPV and NPV for achieving sustained response of this combined HBsAg response were 75% and 85%, respectively.

In the phase III registration trial on peginterferon alfa-2a treatment in HBeAg-positive patients, HBsAg level <1500 IU/ml at week 12 and week 24 during treatment generated PPVs of 57% and 54% and NPVs of 72% and 76%, respectively, for HBeAg seroconversion 6 months post-treatment [51]. This study also demonstrated an HBsAg clearance rate of 17.6% at 6 months post-treatment in patients with HBsAg level <1500 IU/ml at week 12 and HBeAg seroconversion 6 months post-peginterferon treatment. The association of HBsAg and sustained HBeAg seroconversion was confirmed by the results of the NEPTUNE study [52].

Sonneveld *et al.* showed a more vigorous decline in HBsAg level starting at week 4 of peginterferon alfa-2b treatment in responders defined as HBeAg loss with HBV DNA <2000 IU/ml at 26 weeks post-treatment [53]. Patients who did not have any decline in HBsAg at week 12 achieved response in only 3%. Therefore, the NPV of the absence of any decline in HBsAg at week 12 is 97% for prediction of response 6 months post-treatment. However, the retrospective analysis from the phase III trial of peginterferon alfa-2a did not confirm a high NPV as in the Sonneveld study; 18% of patients who did not have any HBsAg decline at week 12 achieved HBeAg loss and HBV DNA <2000 IU/ml at 6 months post-treatment (NPV 82%) [54]. It is possible that differences in study population and HBV genotypes could explain why the high NPV in the Sonneveld study was not confirmed. The Sonneveld study included mainly Caucasian patients (72%) infected with genotype A and D HBV (35% and 39%, respectively), whilst 87% of patients in the peginterferon alfa-2a study were Asians mainly infected with genotype B and C HBV (23% and 71%, respectively). On the other hand, week 12 HBsAg > 20000 IU/ml has an NPV of 84% in the peginterferon alfa-2a phase III study and 100% in the NEPTUNE study for sustained HBeAg seroconversion [51,52].

Suggestions

The decline in serum HBsAg level at weeks 12 and 24 during peginterferon treatment can be used as a surrogate marker to predict sustained response in patients with HBeAg-positive CHB, and to identify patients who are unlikely to have sustained response despite continued treatment. In general, poor HBsAg response at week 12 can predict non-responders and good HBsAg response at week 24 can predict good responders to peginterferon therapy (Table 1).

Challenges

Further studies to identify and validate optimal cutoff levels of HBsAg are needed. Furthermore, HBsAg levels and their declines during peginterferon treatment appear to differ across HBV genotypes, suggesting genotype-specific algorithms are required [55–57]. More research on the management of patients with sub-optimal HBsAg response will be needed before making recommendations.

HBeAg-negative patients

Current evidence

Response rates of HBeAg-negative patients to peginterferon are low, and, therefore, baseline predictors of response are important when considering peginterferon based treatment in these patients. Unfortunately, only one study has so far addressed this

Table 1. Proportion of HBeAg-positive patients with sustained virological response (SVR) as predicted by serum HBsAg at week 12 and 24 of treatment. In general, poor HBsAg response at week 12 can predict non-responders and good HBsAg response at week 24 can predict good responders to peginterferon therapy.

			Prediction at week 12		Prediction at week 24	
Author	[Reference]	HBsAg	% patient	% SVR	% patient	% SVR
Sonneveld	[53]	No decline	31	3	25	8
Piratvisuth	[54]	No decline	24	18	NA	NA
Lau	[51]	>20,000 IU/ml	22	16	13	15
Gane	[52]	>20,000 IU/ml	18	0	14	0
Lau	[51]	<1500 IU/ml	23	57	34	54
Gane	[52]	<1500 IU/ml	27	58	40	57
Chan	[49]	<300 IU/ml	NA	NA	23	62
		<300 IU/ml and >1 log decline	NA	NA	13	75

NA = not available.

clinical problem [58]. One other report of preliminary data from a small cohort of patients treated with peginterferon alfa-2a and adefovir showed that lower baseline HBsAg levels were associated with a sustained virological response (HBV DNA <2000 IU/ml at 6 months post-treatment), but clinically useful cut-off values were not reported [59], and other studies were unable to confirm these findings [60,61].

On-treatment monitoring of HBsAg kinetics in HBeAg-negative patients treated with peginterferon may add considerably to HBV DNA monitoring alone. This is especially relevant since many HBeAg-negative patients treated with peginterferon achieve undetectable HBV DNA levels during therapy, but relapse after discontinuation [62–64]. One relatively small study by Moucari *et al.* reported on 48 HBeAg-negative CHB patients treated with peginterferon alfa-2a for 48 weeks [61]. Similar to HBeAg-positive disease, patients who achieved an off-treatment sustained response (defined as undetectable HBV DNA 6 months after treatment discontinuation) experienced the most pronounced HBsAg declines during treatment. The authors also showed that patients who achieved a decline of more than 0.5 log IU/ml through the first 12 weeks of therapy had a very high probability of response (89%), compared to a probability of only 10% in those who failed to achieve such a decline. Similar findings were reported for a 1 log decline at week 24.

In a retrospective analysis of HBsAg levels during treatment in 120 patients who participated in the registration trial for peginterferon alfa-2a, patients who failed to achieved a decline of at least 10% in serum HBsAg from baseline through 12 weeks of treatment (n = 67) had a lower probability of response (16%, *vs.* 47% in those with a decline >10%, p <0.01) [65]. However, a considerable proportion of patients who did not achieve a 10% decline in HBsAg levels achieved HBsAg loss, limiting the clinical utility of this cut-off as a stopping rule for peginterferon based treatment in HBeAg-negative CHB (Table 2).

Another study among HBeAg-negative patients, predominantly infected with HBV genotype D and treated with peginterferon ± ribavirin, showed that monitoring of both HBV DNA and HBsAg levels during treatment may provide more optimal prediction of response than either marker alone [60]. In this study, patients who failed to achieve both a decline in HBsAg levels as well as a decline of >2 log in HBV DNA did not respond (response defined as HBV DNA <10,000 copies/ml and normal ALT at 6 months post-treatment). Importantly, this possible stopping-rule was recently validated in other cohorts of HBeAg-negative genotype D patients treated for either 1 or 2 years with peginterferon alfa-2a, and its utility was confirmed [66]. However, the stopping-rule has less discriminatory capabilities in HBeAg-negative patients infected with other HBV genotypes. A possible explanation for this discrepancy is the influence of HBV genotype on HBsAg kinetics during treatment, as recently shown in a small study [67], suggesting a need for genotype specific prediction rules.

Table 2. Prediction of treatment response by HBsAg decline at different phases of peginterferon therapy for HBeAg-negative chronic hepatitis B in the phase III trial of peginterferon alfa-2a [56,59].

Time (week)	HBsAg decline	n	Response (n, %)	Definition of response
12	≥10%	53	25 (47)	HBV DNA <4 log at year 1
	<10%	67	11 (16)	
	≥10%	53	12 (23)	HBsAg loss at year 5
	<10%	67	5 (7)	
24	≥10%	67	29 (43)	HBV DNA <4 log at year 1
	<10%	53	7 (13)	
	≥10%	67	15 (22)	HBsAg loss at year 5
	<10%	53	3 (4)	
48	>1 log	43	13 (30)	HBsAg loss at year 3
	≤1 log	155	4 (3)	

In addition to on-treatment prediction of response, one study has investigated the use of end-of-treatment HBsAg levels as predictors of sustained off-treatment response and subsequent HBsAg clearance [68] (Table 2). In this study, patients with HBsAg levels <10 IU/ml (n = 23) at week 48 had a 52% probability of HBsAg clearance through 3 years of post-treatment follow-up, compared to only 2% in all other patients. Importantly, the predictive capabilities of HBsAg levels at week 48 were higher than that of HBV DNA levels; of those patients with undetectable HBV DNA at week 48 (n = 161), only 15% achieved HBsAg clearance. Overall, patients with a HBsAg level >19 IU/ml (or a decline from

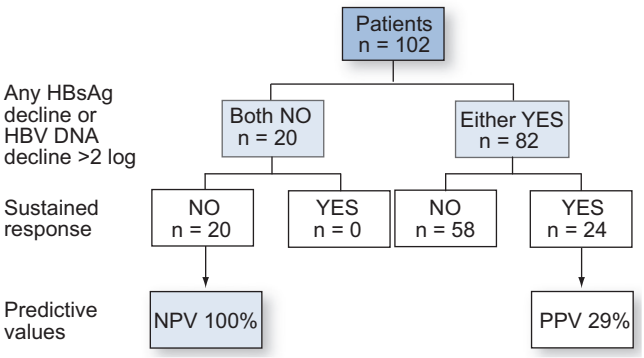

Fig. 3. Flow chart on the use of HBV DNA and HBsAg to predict response to peginterferon treatment in the PARC study.

baseline <0.46 log) had such a low probability of a sustained response through 3 years of post-treatment follow-up that retreatment in these patients with nucleos(t)ide analogues (NA) seems warranted [65].

Suggestions

Combination of HBsAg and HBV DNA decline at week 12 of peginterferon therapy could well be used as a stopping rule in clinical practice for HBeAg-negative patients, particularly among those infected by genotype D HBV (Fig. 3).

Challenges

The early identification of sustained response to peginterferon therapy (high positive predictive value) needs to be improved in HBeAg-negative disease. The use of HBV DNA and HBsAg to guide stopping of peginterferon in genotype B and C HBV infected patients needs to be validated in future studies.

Treatment with nucleos(t)ide analogues

Current evidence

General trend of HBsAg decline on NA therapy

Until recently only few studies have analyzed HBsAg levels during therapy with NA [69–74]. Most studies included heterogeneous patient populations (e.g. transplanted patients [71] or focused only on patients with anti-HBs seroconversion or assessed only few time-points during NA treatment [68].

Some studies have compared HBsAg kinetics between patients treated with interferon alfa and NA [68,70,75]. The data suggest that HBsAg decline during NA therapy is slower and less pronounced compared to interferon treatment, despite a higher effect on HBV DNA suppression. For example, Manesis *et al.* compared HBsAg decline during a median of 33 months of lamivudine treatment and compared it with that observed with interferon [70]. Patients treated with interferon showed a stronger and faster HBsAg decline than lamivudine treated patients. Based on HBsAg kinetics, the authors estimated that the time to HBsAg loss was around 5 years for patients with sustained response to interferon compared with >10 years for lamivudine virological responders. Other studies suggested that the predicted median time to HBsAg loss in NA treated patients is more than 30 years [76,77]. Brunetto *et al.* analyzed 386 HBeAg-negative patients treated either with peginterferon alfa-2a and/or lamivudine. HBsAg decrease was stronger with peginterferon (alone or combined with lamivudine; mean decline at end of treatment, −0.71 and −0.67 log IU/ml, respectively) compared to treatment with lamivudine alone (−0.02 log IU/ml) [68]. The reason for the slow decline of HBsAg in NAs treatment is probably because NAs inhibit only the reverse transcription of the pregenomic RNA 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 interferon leads to a more dramatic decline in HBsAg production and secretion.

All studies investigating quantitative HBsAg during NA therapy suggest that HBsAg decline is slower than that of serum HBV DNA [67], even in patients who subsequently clear HBsAg [71,72]. This may reflect the remaining cccDNA in the infected hepatocytes in patients with long-term established chronic HBV infection. This is different to the situation during acute HBV infection where HBV DNA and HBsAg decline in parallel [78]. Borgniet *et al.* analyzed HBsAg kinetics in 16 patients who cleared HBsAg during antiviral therapy. In most patients HBsAg persisted after HBV DNA elimination from the serum for 1–5 years, with a progressive decline in titer observed. The average time to HBsAg loss after undetectable levels of HBV DNA were achieved was 30 months [72].

HBeAg-positive patients

A very important question is whether it would be possible to predict HBsAg loss using quantitative HBsAg values during NA treatment. Wursthorn *et al.* analyzed quantitative HBsAg in 162 HBeAg positive patients treated with telbivudine for at least 3 years [73]. All patients included had a maintained HBV DNA <60 IU/ml after two years of therapy. Nine patients (6%) developed HBsAg loss through the follow-up of three years. A rapid HBsAg decline of more than 1 log after 1 year of treatment was predictive for HBsAg loss, emphasizing the value of quantitative HBsAg for monitoring response to NA therapy. Similarly, in the pivotal trial of tenofovir, patients who had HBsAg loss, tended to have higher baseline HBsAg level and a more rapid decline in HBsAg level as compared to those who failed to lose HBsAg on 4-year tenofovir treatment [79,80]. Genotype A and D HBV had higher baseline HBsAg and more continuous decline in HBsAg than genotype B and C HBV. In a small study in China, among 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 [74].

HBeAg-negative patients

Among entecavir and tenofovir treated patients, the decline in HBsAg is less pronounced in HBeAg-negative patients than in HBeAg-positive patients [75,79]. In a study in Hong Kong among 53 HBeAg-negative patients on lamivudine treatment for a mean of 19 months, the end-of-treatment HBsAg levels could predict sustained viral suppression (HBV DNA $\leqslant$200 IU/ml) [81]. All five patients who had HBsAg reduction by >1 log to $\leqslant$100 IU/ml (PPV 100%) and 4 of the 8 patients who achieved either HBsAg $\leqslant$100 IU/ml or a reduction of HBsAg by >1 log (PPV 50%) had sustained viral suppression at 12 months post-lamivudine

treatment. On the other hand, all 40 patients who had HBsAg reduction by ⩽1 log to a level >100 IU/ml could not achieve sustained viral suppression at 12 months post-treatment (NPV 100%). The end of treatment HBsAg response could also predict sustained viral suppression and HBsAg loss up to 5 years after stopping lamivudine.

However, in another German study with predominantly HBeAg-negative patients (67%) on NA therapy, early on-treatment decline did not necessarily predict HBsAg loss [78]. Some 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), which is associated with different HBsAg levels [7,8]. Thus, it may represent an already ongoing natural HBsAg decline in some patients. In contrast to interferon therapy [53,55,60,61], early HBsAg kinetics alone during NA therapy may not reliably predict later HBsAg loss. In this German cohort, HBsAg decline of more than 0.5 log in the 2 years after virological response (defined as HBV DNA <100 IU/ml) was associated with later HBsAg loss [78]. However, patients who received no treatment also demonstrated similar HBsAg kinetics during the natural course of HBV infection in this study. This suggests that NA therapy may have only a limited impact on HBsAg suppression, explaining that HBsAg loss remains a rare event during NA therapy. Thus, most patients treated with NA will require life-long maintenance therapy. Taking all currently available data into account, HBsAg loss during NA therapy may be limited to patients with more active immune responses also supported by data showing than NA induced HBsAg loss is associated with higher baseline ALT [76,78].

Suggestions

Overall, serum HBsAg decline is slow and does not correlate with HBV DNA levels during treatment with NAs. However, a rapid serum HBsAg decline during NA therapy, ideally after virological response has been achieved, may identify patients who will clear HBsAg in the long-term. A 6–12 monthly assessment of quantitative HBsAg level can be considered to monitor NA therapy. Among Asian patients (genotype B and C HBV infected patients), an HBsAg level of <100 IU/ml might predict lower risk of relapse and stopping treatment can be considered. This is based on limited evidence and further validation by larger scaled, multi-centered studies will be needed [74,81,82].

Challenges

It will be important to identify a level of serum HBsAg reduction or a cut-off that is associated with subsequent HBsAg loss. It will also be of interest to identify an HBsAg level that is associated with effective immune control where termination of antiviral therapy is possible with low risk of reactivation. Whether this cut-off is in accordance with the one proposed for the identification of low replicative inactive HBsAg carrier remains to be determined [42,43,83]. It is also unknown if viral breakthrough due to drug resistance affects quantitative HBsAg level as mutations in the HBV polymerase can also involve changes in the HBsAg [69]. As certain mutants at the surface region, which overlaps with the polymerase region, might influence the amount of HBsAg level detected by the Architect (Abbott) and Elecsys (Roche) assays, it appears advisable to use the same assay for follow-up of individual patients on treatment [84,85].

Unanswered questions and perspectives

In the recent years, increasing evidence shows that in chronic HBV infection, serum HBsAg levels are indirectly correlated with the control of the infection: the higher the control, the lower serum HBsAg levels. Accordingly, serum HBsAg levels are lower in inactive carriers than in HBeAg-negative CHB patients and decline significantly during effective antiviral treatment. The information provided by serum HBsAg quantification is different but complementary to that of serum HBV DNA. Hence, HBsAg quantification should not be used as a substitute for HBV DNA measures in clinical practice. In particular, among patients on NA therapy, HBV DNA is used to monitor response to treatment and emergence of drug resistance, while HBsAg level may have a role in identifying HBeAg-negative patients suitable for one treatment cessation.

However, until now, a precise biological understanding of these clinical findings is missing: an overall correlation has been suggested between serum HBsAg levels and the intrahepatic amount of cccDNA, but this may not hold true in all the phases of HBV infection, particularly in the HBeAg-negative phase. We may speculate that HBsAg is the indirect expression of cccDNA amounts and transcriptionally active cccDNA, but we need to better understand the complex equilibrium between intracellular HBV biology and the host's immune system and its relations with transcription of HBsAg specific mRNAs, HBsAg production and secretion. Furthermore, we need to know whether HBV heterogeneity in terms of genotypes and "S" gene variability, either naturally occurring or therapy induced, influences serum HBsAg levels in clinically relevant terms. This information is needed to identify correctly HBsAg thresholds to be used in clinical practice.

Substantial variations of serum HBsAg levels in the different phases of HBV infection propose quantitative HBsAg as a new diagnostic tool for the characterization of HBV carriers. However, in spite of the different cut-offs so far identified, larger multicenter studies have to be performed for their appropriate clinical validation. In addition, further work is required to understand whether monitoring serum HBsAg levels at the time of HBeAg to anti-HBe seroconversion and their delta variations over time might help to distinguish patients with durable seroconversion from those with higher risk of HBeAg seroreversion. Accordingly, HBsAg monitoring could provide a useful tool for tailoring the duration of antiviral treatment in HBeAg positive patients. It also remains to be analyzed if HBsAg quantification might serve as an indicator of viral breakthrough in patients who have become HBV DNA negative in serum but remain HBsAg positive. In addition to the issue of successful immune clearance, in HBeAg positive patients, data are missing on the relationship between serum HBsAg level and the severity of histologic grading and staging. Overall, to improve the diagnostic accuracy in the characterization of HBV carriers, more studies must be performed on the combined use of quantitative HBV DNA and HBsAg as their complementary information on the phases of HBV infection may be very useful in clinical practice to define the specific condition of the single HBV carrier during the highly dynamic phases of chronic HBV infection. Finally it will be important to identify levels of HBsAg reduction or cut-offs that are associated with the effective immune control that brings to subsequent HBsAg loss, therefore, allowing termination of NA therapy with the lowest risk of reactivation. Whether this cut-off is in accordance with the one proposed for the identification of low replicative inactive

HBsAg carriers or peginterferon treated patients remains to be determined.

Conflict of interest

H.L.Y.C. is a consultant and advisor of Abbott, Bristol Myers Squibb, F. Hoffmann-la Roche, Merck and Novartis Pharmaceutical. A.T. has served as an advisor to Merck, Roche, Gilead and Janssen-Cilag and has received speaker's fees from Merck and Roche. T.P. served on the advisory board and is on the speaker bureaus for Roche, Schering-Plough Corporation, Novartis, GlaxoSmithKline and Bristol-Myers Squibb. M.C. received lecture fees and/or consult fees from GlaxoSmithKline, Gilead, Novartis, Bristol-Myers-Squibb, Roche, and Merck. M.R.B. is speaker bureau/advisor of Abbott, BMS, Gilead, Hoffmann-la Roche and Merck. H.L.T. received travel support, lecturer fees, grants and consultant fees by Novartis, Roche and/or Abbott, and his wife is an employee of Abbott and holds also stocks in Abbott. J.H.K. is a consultant and advisor of Abbott, Bristol Myers Squibb, GlaxoSmithKline, Merck, Novartis, Omrix, and F Hoffmann-la Roche. J.D.J. is an advisory board member of Bristol Myers Squibb, Glaxo-Smith-Kline, Novartis Pharmaceutical, Merck, and Roche China. H.W. received lecturer/consultant fees, and grant support from Abbott, BMS, Gilead, Merck, Novartis, and Roche. H.L.A.J. received grants from and is consultant for Bristol Myers Squibb, Gilead Sciences, Novartis, Roche, and Merck. P.M. is a consultant for, and is on the speakers' bureau of Roche, Schering-Plough, Gilead, Bristol-Myers Squibb, GlaxoSmithKline, and Idenix-Novartis; a consultant for and advises Vertex, Valeant, Human Genome Sciences, Cythesis, Intermune, Wyeth, and Tibotec; and an advisor for Coley Pharma. M.M.-P. and S.L. reported no potential conflict of interest.

Financial support

F. Hoffmann-la Roche has provided unrestricted financial support for the meeting of the group but did not advise nor interfere with the content of this paper.

Appendix. Good Practice in using sAg in Chronic Hepatitis B Study Group (GPs-CHB Study Group)

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