Use of Quantitative Serum HBsAg for Optimization of Therapy in Chronic Hepatitis B Patients Treated with Pegylated Interferon Alfa-2a: a Romanian Cohort Study

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ABSTRACT

Background & Aims: The aim of the study was to assess the clinical utility of serum HBsAg quantification as a surrogate biomarker for the prediction of sustained virological response (SVR) in chronic hepatitis B (CHB) patients treated with Pegylated Interferon alfa-2a (Peg-IFN α-2a).

Methods: We performed a prospective cohort study which included 57 patients with CHB treated 48 weeks with Peg-IFN α-2a and followed for another 24 weeks. HBsAg was quantified at the baseline, during treatment and at the end of follow-up. SVR was defined as HBV-DNA below 2,000 IU/ml at 24 weeks after the end of therapy.

Results: The majority of patients had HBeAg-negative CHB (68%, n=39). Positive predictive factors for SVR at baseline were low levels of HBsAg (3.72 log10 IU/ml, p=0.032) and HBV-DNA (3.96 log10 IU/ml, p=0.035). During treatment, patients who achieved SVR showed a marked decrease in serum HBsAg in comparison with nonresponders (at week 48 mean decrease of 1.06 ± 1.3 log10 IU/ml versus 0.04 ± 0.5 log10 UI/ml, p=0.005). On therapy, HBV-DNA reduction ≥ 2 log10 IU/ml with any decrease of HBsAg level at week 12 had a positive predictive value (PPV) of 80% (95% CI: 51.91–95.43%) for SVR, while HBV-DNA decline < 2 log10 IU/ml without any decline of HBsAg had a negative predictive value (NPV) of 85.71% (95% CI: 42.23–97.63%) for SVR.

Conclusions: HBsAg quantification combined with HBV-DNA assessment could become an early useful tool to optimize the management of CHB patients treated with Peg-IFN α-2a, according to response guided therapy.

Key words: quantitative HBsAg – chronic hepatitis B – HbsAg

INTRODUCTION

Worldwide hepatitis B virus (HBV) infection has a high prevalence (350–400 million people are chronic HBV surface antigen carriers) and also an increased morbidity and mortality because of end-stage liver disease (0.5–1 million deaths annually) and limited possibilities of therapy [1].

Chronic hepatitis B (CHB) remains a difficult to treat disease because, at this time, no treatment provides both an optimal virological and immunological control, there is a high rate of relapse following any antiviral therapy and there are no therapy stopping-rules, especially in hepatitis B e antigen (HBeAg) negative patients treated with nucleoside or nucleotide analogues (NAs) [2].

Today’s major interest in chronic infection with HBV is the quantitative evaluation of hepatitis B surface antigen (HBsAg) [3]. Since its discovery by Blumberg in 1965, HBsAg is the first serological marker used for HBV infection diagnosis [4]. Loss of serum HBsAg, spontaneous or induced by treatment, represents the best possible outcome in HBV-infected patients [5]. However, this ideal end point of therapy is poorly / rarely achieved with currently available anti-HBV agents [1]. The interest in HBsAg quantification as a surrogate marker for prognosis and treatment response started with the possible correlation between serum
HBsAg and the transcriptional activity of covalently closed circular (ccc) DNA [4]. In the last years several published studies addressed the role of serum HBsAg quantification in predicting treatment response in CHB patients treated mainly with Pegylated Interferon (Peg-IFN) α-2a. Some authors have recently proposed an early stopping rule using the combination between serum HBsAg and HBV DNA levels for HBeAg negative CHB patients treated with Peg-IFN α-2a [2].

The aim of the present study was to assess the clinical utility of serum HBsAg quantification as a surrogate biomarker for the prediction of SVR in the early phase of treatment in a cohort of CHB patients treated with Peg-IFN α-2a in Romania. Secondary objectives of the study were the assessment of serum HBsAg kinetics during and after cessation of therapy, as well as the evaluation of biochemical, serological and virological response in this category of patients.

METHODS

Patient population

Fifty-seven consecutive patients diagnosed with CHB by presence of serum HBsAg for at least 6 months prior to enrolment, regardless of HBeAg status, were included in this cohort study. The patients were recruited in two reference university hospitals in Romania: the National Institute of Infectious Diseases Prof Dr Matei Bals, Bucharest (n=25) and the Infectious Diseases Hospital Dr Victor Babes, Timisoara (n=32). All enrolled patients fulfilled the following inclusion criteria: treatment-naïve or experienced CHB patients (but who did not respond or relapsed after a previous course of IFN or NAs and who stopped the therapy for at least 6 months), over 18 years old, alanine aminotransferase (ALT) level below 1 to 10 times the upper limit of normal (ULN), HBV DNA above 2,000 IU/ml and negative pregnancy test for female patients. No patient had evidence of cirrhosis, hepatocellular carcinoma or HCV, HDV or HIV coinfection. Patients were treated with Peg-IFN α-2a at a dose of 180 /μg/week for 48 weeks and followed for another 24 weeks. In order to assess the virological response we used the following definitions: end of treatment response (EOT) was defined as HBV DNA under 2,000 IU/ml at the 48 weeks of treatment; sustained virological response (SVR) was defined as HBV DNA under 2,000 IU/ml at the end of follow-up (EOF); relapse was defined as HBV DNA under 2,000 IU/ml at the EOT and a subsequent increase of serum HBV DNA above this threshold within the 24 weeks of follow-up; nonresponse was defined as HBV DNA above 2,000 IU/ml at EOT; biochemical response was defined as normalisation of ALT levels and was evaluated at EOT and EOF; serological response was defined as HBeAg loss and serocconversion to anti-HBe antibodies (for HBeAg positive patients) and HBsAg loss with/without development of anti-HBs antibodies (regardless of HBeAg status) and it was also evaluated at end of treatment (EOT) and end of follow-up (EOF).

The study was approved by the Hospitals’ Ethics Committees.

Laboratory investigations

HBV genotype was not evaluated since there was already available data regarding HBV genotype prevalence in Romania (79.21% - genotype D, 12.87% - genotype A, the rest of the patients - other genotypes or mixed genotypes: F, A/F, D/E, D/F) [6]. A priori, we considered patients to have the same profile of genotype as in the above study [6], the patients being selected from two representative regions of the country (south-east for Bucharest and west for Timisoara).

Alanine aminotransferase assessment was performed in the local hospital laboratories; the values were expressed in IU/ml. This parameter was checked during therapy (weeks 0, 4, 12, 24, 48) and during follow-up (week 72).

Serum HBV DNA was measured using two types of real-time polymerase chain reaction (RT-PCR) assays: m2000sp/m2000rt (Abbott Reactives; detection limit of 15 IU/ml) and COBAS TaqMan (Roche Diagnostics; lower limit of detection of 20 IU/ml). As well as other parameters, HBV DNA was assessed during therapy (weeks 0, 4, 12, 48) and during follow-up (week 72).

Quantification of serum HBsAg was performed at the same interval as ALT using the commercial test ARCHITECT 2000 (Abbott Architect 2000 HBsAg assay). The test has a dynamic range of 0.05 – 250 IU/ml with a dilution protocol according to the attached manual for samples with HBsAg concentration outside of the standard range.

All tests results are given in IU/ml.

Statistical analysis

Because this study is based on a real life concept, we performed an intent to treat (ITT) analysis which included all patients who received at least one dose of medication (Peg-IFN α-2a) even if they could not be evaluated for various reasons at week 72.

We used the Mann-Whitney U test to analyse differences between groups for continuous variables and the chi-square or Fisher’s exact test for dichotomic variables. Significance level was set at α = 0.05. Statistical analysis was performed using SPSS v17.0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA).

RESULTS

Baseline characteristics

Baseline characteristics of the 57 patients are shown in Table 1. Forty-six patients were male (80%). Median age was 34 years (IQR, 24-41.5 years). The median value of ALT was 78 IU/ml (IQR, 38.5-133 IU/ml). The median value of serum HBV DNA was 4.9 log10 IU/ml (IQR, 3.6-6.4 log10 IU/ml). The median value of serum HBsAg was 4.1 log10 IU/ml (IQR, 3.7-4.4 log10 IU/ml). Most patients were HBeAg negative (n=39, 68%).

By univariate analysis at baseline (Table I), we found that SVR was associated with low baseline level of serum HBsAg (median value of 3.7 log10 IU/ml [IQR, 3.1-4.1 log10 IU/ml] versus 4.1 log10 IU/ml [IQR, 3.9-4.3 log10 IU/ml], p=0.032). SVR was also associated with low baseline level of serum HBV DNA (median value of 3.9 log10 IU/ml [IQR, 3.5-5.5 log10 IU/ml] versus 5.8 log10 IU/ml [IQR, 4.3-6.8 log10 IU/ml], p=0.033). The rest of the baseline parameters (age, gender, ALT, HBeAg status) did not differ between patients who achieved SVR and those without SVR.
Quantitative serum HBsAg in chronic hepatitis B patients under therapy

Table 1. Baseline characteristics of the enrolled patients (divided according to SVR)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=57)</th>
<th>SVR+ (n=22)</th>
<th>SVR- (n=16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years [median (IQR)]</td>
<td>34 (24-41.5)</td>
<td>35.5 (28.7-42.2)</td>
<td>37.5 (23.2-45.7)</td>
<td>0.966</td>
</tr>
<tr>
<td>Male gender [n(%)]</td>
<td>46 (80)</td>
<td>15 (68)</td>
<td>14 (87)</td>
<td>0.254</td>
</tr>
<tr>
<td>ALT, IU/ml [median (IQR)]</td>
<td>78 (38.5-133)</td>
<td>60 (28.7-110)</td>
<td>83.5 (40.5-203.5)</td>
<td>0.153</td>
</tr>
<tr>
<td>qHBsAg, log IU/ml [median (IQR)]</td>
<td>4.1 (3.7-4.4)</td>
<td>3.7 (3.1-4.1)</td>
<td>4.1 (3.9-4.3)</td>
<td>0.032</td>
</tr>
<tr>
<td>HBV-DNA, log IU/ml [median (IQR)]</td>
<td>4.9 (3.6-6.4)</td>
<td>3.9 (3.5-5.5)</td>
<td>5.8 (4.3-6.8)</td>
<td>0.033</td>
</tr>
<tr>
<td>HBeAg, [n(%)]</td>
<td>18 (32)</td>
<td>3 (14)</td>
<td>4 (25)</td>
<td>0.425</td>
</tr>
<tr>
<td>HBeAb, [n(%)]</td>
<td>39 (68)</td>
<td>19 (86)</td>
<td>12 (75)</td>
<td>0.425</td>
</tr>
</tbody>
</table>

Data are expressed as the median and as percentages. Abbreviations: IQR: interquartile range; SVR+: presence of SVR; SVR-: absence of SVR

Virological response

Of all 57 patients, 37 (64.9%) developed EOT virological response and 22 (38.6%) achieved SVR. Serum HBV DNA kinetics during the therapy period and follow-up according to treatment response is showed in Fig. 1. The mean HBV DNA decline was stronger during the early phase of therapy (weeks 1 to 12) in patients who achieved SVR compared to those without SVR (3.6 ± 1.2 log10 IU/ml versus 1.8 ± 1.7 log10 IU/ml, p=0.003) (Fig. 1). The profile of HBV DNA decline was similar in patients who achieved SVR and in patients who developed relapse following an initial EOT response. Still, the mean value of viral load at 12 weeks of treatment was significantly lower in patients who achieved SVR in comparison with relapsers (Fig. 2).

Serum HBsAg kinetics

Overall, HBsAg level decreased significantly through the 48 weeks course of therapy (the mean decline was 0.48 log10 IU/ml, p=0.003). However, patients who obtained SVR experienced a significantly more important decrease in serum HBsAg titer (Fig. 3). The mean declines of serum HBsAg in this subset of patients at 12, 24 and 48 weeks were 0.5 ± 1 log10 IU/ml, 0.6 ± 1 log10 IU/ml and 1 ± 1.3 log10 IU/ml, respectively. On the contrary, the non-responder patients did not achieve a significant decrease in serum HBsAg level during treatment (the mean decline at week 48 was 0.04 ± 0.5 log10 IU/ml). In fact, the mean level of HBsAg was almost the same as the baseline value in this category of patients (Fig. 3). In patients who developed EOT response, the profile of serum HBsAg kinetics was different in patients who achieved SVR compared to those who relapsed. Thus, the serum HBsAg level did not decline during treatment in relapsers, having the same profile as nonresponder patients (Fig. 4).
Serological response

Of all 18 HBeAg positive patients, 3 patients (16%) and 4 patients (22%), respectively, developed seroconversion to anti-HBe at EOT and EOF.

Regarding the rate of seroconversion to anti-HBs, 1 patient (1.7%) and 3 patients (5.2%), respectively, achieved this ideal outcome at EOT and EOF.

Biochemical response

The evolution of ALT levels during therapy was different in patients who achieved SVR in comparison with nonresponders. Thus, in patients with SVR, ALT levels increased significantly in the first 12 weeks, followed by a progressive decline during the remaining treatment period and follow-up (Fig. 5).

Prediction of virological response

From a practical point of view, it is already well known that early identification of patients with the highest chance of treatment response and in particular of those with the lowest chance of response after 48 weeks course of Peg-IFN α-2a therapy, has the greatest clinical relevance.

To identify the patients with a good chance of response and mainly those with the lowest chance of treatment response, we chose to use the PARC “rule” (the presence of any HBsAg decline and/or HBV DNA decline ≥ 2 log10 IU/ml at week 12 of therapy) [2]. We found that HBV-DNA reduction more than 2 log10 IU/ml combined with any decrease of HBsAg level at week 12 on therapy has a positive predictive value (PPV) of 80% (95% CI: 51.91–95.43%) for SVR, while HBV-DNA decline < 2 log10 IU/ml without any decline of HBsAg has a negative predictive value (NPV) of 85.71% (95% CI: 42.23–97.63%) for SVR (Fig. 6).

DISCUSSION

This study is the first prospective study reporting our experience in defining the clinical utility of quantitative HBsAg in CHB patients treated with Peg-IFN α-2a for 48 weeks.

The clinical relevance of HBsAg serum level arises from the correlation with the intrahepatic amount and transcriptional activity of cccDNA, the main replicative template of HBV [4, 7, 8].

HBsAg can be considered a surrogate marker of immune control of HBV during antiviral therapy, regardless of virological response assessed by serum HBV DNA quantification [7]. A HBV DNA decline directly reflects a reduction of viral replication while serum HBsAg decline signifies a reduction of translation of messenger RNA resulted from transcriptional activity of intranuclear cccDNA and integrated DNA sequences [8, 9]. The studies published by different groups of authors have demonstrated that serum levels of HBsAg and HBV DNA vary during the natural history of chronic HBV infection; the highest values were recorded in the “immune tolerant” phase (5.0 log10 IU/ml for HBsAg and 7.5–8.5 log10 IU/ml for HBV DNA) [8, 10, 11]. HBsAg level is reduced in “immune clearance” phase (medium level of 3.0–4.0 log10 IU/ml) and progressively and slowly decreases in patients with persistent normal transaminases after HBeAg seroconversion [10]. The lowest values of HBsAg and HBV DNA were recorded in inactive carrier status, which is also characterized by a high value of HBsAg/HBV DNA ratio [8, 11]. It is well known that there are significant differences in the level of serum HBsAg according to HBV genotype. Patients with genotype A and D have the highest mean value of serum HBsAg (about 4.5 log10IU/ml), compared to genotype B (4.3 log10 IU/ml) and C patients (3.8 log10 IU/ml) [12, 13].

![Fig. 5. Kinetics of ALT during the treatment period and follow-up according to treatment response.](image-url)
One study published by Italian authors in 2010 pointed that in CHB genotype D patients, combining a single-point determination of HBsAg < 1,500 IU/ml and HBV DNA < 2,000 IU/ml may identify "true inactive carriers" with a PPV of 87.9% and a NPV of 96.7% [14]. As a result of sustained immune control, the "inactive carrier" status is associated with a favorable long-term prognosis with a low risk of cirrhosis and HCC in most patients [1]. Therefore, for our study we defined SVR by a decrease of HBV DNA level < 2,000 IU/ml at 48 weeks of treatment with Peg-IFN α-2a and maintaining this response at least 24 weeks after treatment.

In view of already published data on the prevalence of HBV genotypes in Romania that support the dominance of genotype D (about 80%) [6], we did not consider it cost effective to determine the viral genotype for this cohort of patients.

As discussed in other studies, after the initiation of sometimes difficult to tolerate antiviral therapy (even for 48 weeks), it is important to identify as early as possible patients with little chance of response to therapy and those with a high risk of relapse after cessation of treatment [15]. This strategy can lead to avoiding unnecessary continuation of therapy in certain categories of patients with an unfavorable profile for SVR achievement.

There are a series of already known host and virus pre-treatment factors associated with a high rate of virological and serological response to interferon therapy. In our study, univariate analysis of baseline parameters showed that lower mean HBsAg levels (median value 3.7 log10 IU/ml vs 4.1 log10 IU/ml, p = 0.032) and HBV DNA levels (median value 3.9 log10 IU/ml vs 5.8 log10 IU/ml, p = 0.033) were associated with SVR. Although the p value is statistically significant, the results are close to the threshold of 0.05, which can be explained by the relatively small number of patients enrolled in this study. In the published literature, results of a study on HBeAg-positive patients pointed that HBsAg levels < 10 000 IU/ml at baseline were associated with a higher rate of response to peginterferon therapy [16], while other studies, both in HBeAg positive and negative patients, did not confirm this observation [8].

When comparing the treatment with interferon and NAs, it has already been shown that interferon induce a more significant decrease in HBsAg serum levels [8]. Current guidelines position PegIFN among the first therapeutic options due to increased rates of sustained response after a finite course of treatment compared with NAs (20% versus less than 5% in HBeAg positive patients treated for 12 months with Peg-IFN or NAs, respectively) [1, 3, 5]. However, a major disadvantage of PegIFN therapy is the considerable risk of relapse after the 48 weeks of treatment [17, 18]. Due to the absence of useful clinical predictive markers for interferon treatment response and the need for individualization of therapy according to response, there has been an increased interest in on-treatment quantification of serum HBsAg as a possible marker of interferon immunomodulatory effect [2]. Previously published studies underlined that in patients treated with Peg-IFN α-2a serum HBsAg decline during therapy appears to be an important marker for predicting response to treatment [7].

In our study, the average decrease of quantitative HBsAg level during treatment was 0.48 log10 IU/ml, p = 0.003. HBsAg kinetics was different in non-responders patients versus those who achieved SVR. Thus, in non-responder patients there was a lack of HBsAg decline during the 48 weeks of treatment compared to responders, despite a significant decrease in serum HBV DNA in both groups (Fig. 3). One interesting finding of our study showed that the pattern of HBsAg decline was different in patients who achieved virological response at week 48 but later developed relapse, compared to those who maintained their response. In relapers the decrease in serum HBsAg during treatment was minor compared with the significant decrease of HBsAg levels recorded in patients who achieved SVR. Basically, relapers had the same HBsAg declining profile as non-responder patients (Fig. 4). These observations reinforce the idea that HBsAg can be considered a surrogate biomarker for immunological control of HBV infection.

"PARC rule" has been recently validated and published as a stopping rule at week 12, using HBsAg and HBV DNA for HBeAg-negative, genotype D patients treated with Peg-IFN α-2a. According to this rule, we can identify early, with a NPV of 100%, all CHB, HBeAg-negative, genotype D patients that will not achieve SVR after 48 or 96 weeks of treatment with Peg-IFN α-2a [2]. The difference between the results obtained in our study (a NPV of 86% for absence of SVR and a PPV of 80% for those who achieved SVR) and the results published in the validation trial conducted by Rijckborst et al, can be explained by the inclusion in statistical analysis of both types of patients, HBeAg positive and negative, and no stratification of patients according to viral genotype.

All these results set a new standard for therapeutic strategies in CHB, similar to HCV strategies, the standard of response guided therapy. Basically, the combined use of HBsAg and HBV DNA assessment in patients with CHB treated with Peg-IFN α-2a can guide the clinician to evaluate the chances of treatment response with the possibility to individualize therapy strategies, such as "continue and follow-up", "add-on, switch or stop and follow-up".

Obviously, our study has certain limits that may be, at least partially, responsible for some results discordant to those already published in other studies: the small number of enrolled patients (n = 57), the absence of viral genotype evaluation as non-responder patients (Fig. 4), and the results published in our study (a NPV of 86% for absence of SVR and a PPV of 80% for those who achieved SVR) and the results published in the validation trial conducted by Rijckborst et al, can be explained by the inclusion in statistical analysis of both types of patients, HBeAg positive and negative, and no stratification of patients according to viral genotype.

**CONCLUSION**

Our study performed in a Romanian cohort showed, similar to other international published studies, that serum HBsAg quantification combined with HBV-DNA assessment at week 12 of therapy could become an early useful tool to optimize the management of CHB patients treated with Peg-IFN α-2a, according to response guided therapy.

**Conflicts of interest:** Valeriu Gheorghita has served as speaker for Hoffman-La Roche, Bristol-Myers Squibb and Merck Sharp&Dohme; Florin Al Caruntu is speaker and served on the advisory board for Hoffman-La Roche, Bristol-Myers Squibb, Merck Sharp&Dohme, Gilead, Janssen-Cilag, Abbott and GlaxoSmithKline; Manuela Cucescu is speaker for Hoffman-La Roche and Bristol-Myers Squibb.
and served on the advisory board for Hoffman-La Roche, Bristol-Myers Squibb, Merck Sharp&Dohme; Ioana Olaru, Monica N Radu: no conflicts to declare; Gabriel Coltan has served as speaker for Hoffman-La Roche and Bristol-Myers Squibb; Adrian Streinu-Cercel is speaker and served on the advisory board for Hoffman-La Roche, Bristol-Myers Squibb, Merck Sharp&Dohme, Gilead, Janssen-Cilag, Abbott and GlaxoSmithKline.

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