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Original Article

Comparison between quantitative hepatitis B surface antigen, hepatitis B e-antigen and hepatitis B virus DNA levels for predicting virological response to pegylated interferon-α-2b therapy in hepatitis B e-antigen-positive chronic hepatitis B

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Aim: The aim of this study was to compare the clinical applicability of quantitative serum hepatitis B surface antigen (HBsAg), hepatitis B e-antigen (HBeAg) and hepatitis B virus (HBV) DNA for predicting virological response (VR) to pegylated interferon (PEG-IFN) therapy.

Methods: Thirty HBeAg-positive chronic hepatitis B patients who received PEG-IFN-α-2b for 48 weeks were enrolled. Quantitative HBsAg, HBeAg and HBV DNA were measured before, during and after the therapy. Paired liver biopsies were performed before and after treatment for covalently closed circular (ccc)DNA and intrahepatic HBV DNA analysis.

Results: VR at 48 weeks post-treatment, defined as HBeAg seroconversion and HBV DNA less than 10 000 copies/mL was achieved in 10 (33.3%) patients. Responders had significantly lower baseline HBsAg, HBeAg, cccDNA and intrahepatic HBV DNA levels than non-responders. Baseline and reduced levels of $\log_{10}$ HBsAg and $\log_{10}$ HBeAg correlated well with those of $\log_{10}$ cccDNA and $\log_{10}$ total intrahepatic HBV DNA. Responders showed consistent decrease in serum HBsAg, HBeAg and HBV DNA levels during therapy. HBeAg level of 2.0 $\log_{10}$ sample to cut-off ratio at week 24 on therapy provided the best prediction of sustained virological response, with sensitivity and negative predictive values of 85% and 92%, respectively. One patient (3.3%) who cleared HBsAg at follow up exhibited a more rapid decline in serum HBsAg during therapy than those who developed VR without HBsAg clearance.

Conclusion: Quantitative measurement of serum HBeAg during therapy may be superior to serum HBsAg and HBV DNA as a prediction of HBeAg seroconversion. Kinetics of HBsAg levels on therapy may help predict HBsAg clearance after treatment.

Key words: covalently closed circular DNA, hepatitis B e-antigen, hepatitis B surface antigen, hepatitis B virus DNA, quantification, pegylated interferon, virological response.

INTRODUCTION

CHRONIC HEPATITIS B virus (HBV) infection is a major global health problem.[1] Long-term follow-up studies have shown that sustained responders to antiviral therapy have a significant reduction in HBV-related complications including cirrhosis and hepatocellular carcinoma (HCC). The approved antiviral agents for chronic hepatitis B are nucleoside/nucleotide analogs (NA) and α-interferon (IFN-α)-based therapies.[2] IFN acts mainly as an immunomodulator and enhances the cell-mediated immune response in the process of clearing the virus. Thus, response to IFN-based therapies tends to be more sustained than to NA.[2] Currently, pegylated interferon (PEG-IFN) has replaced its conventional form as it is more convenient and has better therapeutic efficacy. However, PEG-IFN therapy can result in only 30–40% hepatitis B e antigen (HBeAg) seroconversion, which is still far from satisfactory.[3]

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Considering the treatment is expensive and has potential side-effects, it is important to identify pretreatment and on-treatment parameters for predicting response and non-response of patients treated with PEG-IFN.

Although quantification of serum HBV DNA remains the most widely used virological marker in the management of patients with chronic hepatitis B, its measurement is costly and may not always represent a reliable indicator of sustained response to antiviral therapy.[4] Increasing evidence has shown that quantitative assays for (HBsAg) and HBeAg may be valuable in identifying patients likely to respond to PEG-IFN therapy.[5–9] For example, baseline HBsAg titer, which is correlated with intrahepatic covalently closed circular (ccc)DNA, is considered a potential predictor of virological response (VR) to combined PEG-IFN and lamivudine treatment in HBeAg-positive hepatitis.[6] In addition, quantification of serum HBeAg before and during therapy may be a useful marker for predicting VR in HBeAg-positive patients treated with PEG-IFN.[7] These data provide essential clues on the importance of measuring HBsAg and HBeAg levels in HBeAg-positive hepatitis during treatment with PEG-IFN, which could offer a new paradigm for predicting treatment response and determining treatment cessation. However, direct comparison between quantitative measurements of HBsAg, HBeAg and HBV DNA for predicting VR to PEG-IFN therapy has never been performed. Thus, the aims of this study were to directly compare the clinical applicability of measuring quantitative HBsAg, HBeAg and HBV DNA before and during PEG-IFN therapy for prediction of VR in patients with HBeAg-positive chronic hepatitis B.

METHODS

Patients

THIRTY PATIENTS WITH HBeAg-positive chronic hepatitis B (23 men and seven women) who had completed the treatment with PEG-IFN-α-2b (Shering-Plough, Kenillworth, NJ, USA) and had been followed up between August 2005 and January 2008 at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, were retrospectively investigated. The patients were between 22 and 54 years of age and fulfilled the following criteria: seropositive for HBsAg and HBeAg, elevation of serum alanine aminotransferase (ALT) for at least 6 months and detectable serum HBV DNA. Paired liver biopsies were performed before and at the end of treatment for histology and intrahepatic viral DNA analysis. The liver histology was graded by the histological activity index (HAI) according to the criteria of Knodell *et al.*[10] which comprise two major components, namely, necroinflammation (HAI-I) and fibrosis (HAI-F).

Pegylated IFN-α-2b was administrated s.c. at a dose of 1.5 μg/kg weekly for 48 weeks. All patients were followed up for an additional 48 weeks after treatment (week 96) to assess VR. VR was defined as HBeAg seroconversion and sustained inhibition of viral replication (HBV DNA level <10 000 copies/mL) until 12 months post-treatment.[11]. Patients without VR were defined as non-responders. According to these criteria, 10 (33.3%) and 20 (66.7%) were classified as responders and non-responders, respectively. Among the responders, HBsAg clearance was achieved in one patient. Thus, the rate of HBsAg clearance in this study was 3.3%.

Serum samples were collected from each patient at baseline, during therapy (weeks 12, 24, 36 and 48) and during follow up (weeks 72 and 96) and stored at −70°C until further tests were performed. Pre- and post-treatment liver biopsy specimens were stored at −70°C until analysis. All patients had been informed as to the study's purpose and had given their written consent.

Serological and virological assays

Qualitative HBsAg, HBeAg and anti-HBe measurements were carried out using a commercially available enzyme-linked immunosorbent assay kit (Abbott Laboratories, Chicago, IL, USA). The quantification of HBsAg was performed using the ARCHITECT *i*2000SR (Abbott Diagnostic, Chicago, IL, USA) according to the manufacturer's specifications.[12] The sensitivity of the assay ranged 0.05–250 IU/mL. Samples with HBsAg titers beyond the upper range were diluted with phosphate buffered saline (PBS) into 1:10, 1:1000 and 1:10 000 prior to further analysis.

The quantification of HBeAg was performed using the ARCHITECT *i*2000SR (Abbott Diagnostic), a two-step immunoassay based on the use of chemiluminescence microparticles (CIMA), according to the manufacturer's instructions.[13] Briefly, undiluted samples were mixed with paramagnetic beads coated with anti-HBe antibodies. HBeAg in the sample then attached to the magnetic beads through the presenting antibodies. After a washing step, a conjugate and reactant were added leading to the emission of light, which was proportional to the determined HBeAg concentration. The assay was calculated based on the ratio of the sample relative light unit (RLU) to the cut-off RLU (S/CO) for each specimen. Samples with S/CO values more than 1.0 were considered positive results for HBeAg.

Hepatitis B virus DNA was extracted from 100 μL serum with proteinase K in lysis buffer followed by phenol/chloroform/isoamyl alcohol extraction. The DNA pellet was dissolved in 30 μL sterile distilled water. Of the resuspended HBV DNA solution, 1 μL was subjected to quantification of HBV DNA by real-time polymerase chain reaction (PCR) using SYBR Green I fluorescent dye as previously described.[14] The HBV genotypes were determined by direct sequencing on the surface gene, as previously described.[14]

Quantification of cccDNA and intrahepatic HBV DNA

Approximately 5–10 mg of liver biopsy specimens taken at baseline and end of treatment were studied. Liver tissue was incubated overnight in lysis buffer with proteinase K followed by phenol/chloroform/iso-amyl alcohol extraction. Total intrahepatic HBV DNA was determined by real-time PCR using SYBR Green I fluorescent dye, applying the same conditions as specified for the measurement of serum HBV DNA.

Hepatitis B virus cccDNA was quantitatively determined as described previously, with some modifications.[15] Briefly, the reaction mixture comprised 1.0 μL of DNA sample, 6.0 μL 2.5X MasterMix solution, 0.3 μL of 25 mM magnesium solution (Mastermix; 5 PRIME, Hamburg, Germany), 0.75 μL of 25 μM forward primer, 0.75 μL of 25 μM reverse primer, 0.24 μL of 10X SYBR Green (QIAGEN, Hilden, Germany) and distilled water to a final volume of 15 μL. Real-time PCR amplification was carried out in a LightCyCler (Roach, Basel, Switzerland). After a pre-incubation step at 95°C for 10 min in order to activate the Taq polymerase, amplification was performed during 40 cycles including denaturation (94°C, 15 s), annealing (59°C, 30 s) and extension (72°C, 60 s). A single fluorescent signal was obtained once per cycle at 80°C after extension step.

To standardize the extracted DNA from liver tissue in term of copies per genome equivalent, the amount of the β-globin gene was measured. Primer sequences for the β-globin gene were describe previously.[16] PCR was performed in 10 μL reaction volumes containing 1 μL of DNA, 5 μL of 2.5X Mastermix solution, 0.1 μL of 25 μM forward primer, 0.1 μL of 25 μM reverse primer, 0.25 μL of 10X SYBR Green and distilled water. Amplification was performed for 3 min at 94°C, 40 cycles of 10 s at 95°C for denaturation, 15 s at 60°C for annealing and 20 s at 72°C for extension. The fluorescence intensity of the PCR products was measured at 78°C. A standard curve was created the same as the quantification of HBV DNA by using pGemT-Easy Vector inserted with the amplicon.

Ethical considerations

The study was in accordance with the principles of the 1975 Declaration of Helsinki and approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University.

Statistical analysis

Data were expressed as mean ± standard deviation (SD), and percentages as appropriate. Comparisons between groups were analyzed by the c2 or Fisher's exact test for categorical variables and by the Mann–Whitney *U*-test or Student's *t*-test for quantitative variables. Pearson's correlation coefficient was tested for correlation between two variables. Area under the receiver–operator curve (ROC) was calculated to assess the predictive values of variables for VR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated in accordance with standard methods. $P < 0.05$ were considered statistically significant. Data were analyzed using the SPSS software for Windows ver. 14.0.

RESULTS

Baseline characteristics

BASELINE CHARACTERISTICS OF the patients are shown in Table 1. Responders had significantly lower baseline $\log_{10}$ HBsAg, $\log_{10}$ HBeAg, $\log_{10}$ cccDNA and $\log_{10}$ total intrahepatic HBV DNA levels than non-responders. No significant difference between groups was observed in respect to age, sex, pretreatment ALT level, HBV genotype, necroinflammatory and fibrosis scores. Lower HBV DNA levels at baseline tended to predict VR; however, the difference between groups did not reach statistical significance.

Pretreatment $\log_{10}$ HBsAg showed positive correlation with both $\log_{10}$ HBeAg ($r = 0.516$, $P = 0.003$) and $\log_{10}$ (serum HBV DNA) ($r = 0.633$, $P < 0.001$), as well as with $\log_{10}$ cccDNA ($r = 0.517$, $P = 0.008$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.635$, $P = 0.001$). $\log_{10}$ HBeAg displayed correlation with $\log_{10}$ (serum HBV DNA) ($r = 0.513$, $P = 0.004$) and $\log_{10}$ cccDNA ($r = 0.507$, $P = 0.010$), but not with $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.362$, $P = 0.076$). $\log_{10}$ (serum HBV DNA) was not significantly correlated with $\log_{10}$

Table 1 Characteristics of responders and non-responders at baseline

	Responder ($n = 10$)	Non-responder ($n = 20$)	P
Age, year	37.0 ± 9.4	36.7 ± 8.2	0.921
Sex, male	7 (70%)	16 (80%)	0.657
ALT level, U/L	96.6 ± 65.6	90.9 ± 71.3	0.852
HBV genotype			0.333
B	1 (10%)	5 (25%)	
C	9 (90%)	15 (75%)	
Serum HBsAg level, $\log_{10}$ IU/mL	3.46 ± 0.47	4.01 ± 0.64	0.013
Serum HBeAg level, $\log_{10}$ S/CO	2.22 ± 0.52	2.73 ± 0.54	0.023
Serum HBV DNA level, $\log_{10}$ copies/mL	5.93 ± 0.67	6.43 ± 0.93	0.107
cccDNA level, $\log_{10}$ copies/genome equivalent	0.28 ± 0.75	1.46 ± 0.74	0.001
Total intrahepatic HBV DNA level, $\log_{10}$ copies/genome equivalent	1.19 ± 0.36	2.10 ± 0.69	0.001
Necroinflammatory score	3.8 ± 1.6	4.2 ± 1.8	0.563
Fibrosis score	1.1 ± 0.8	1.2 ± 0.9	0.719

ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

cccDNA ($r = 0.295$, $P = 0.152$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.347$, $P = 0.089$).

End of treatment characteristics

At end of treatment, responders had significantly lower serum HBsAg, HBeAg, HBV DNA, cccDNA and intrahepatic HBVDNA levels than non-responders. There was a tendency towards lower ALT, necroinflammatory and fibrosis scores in responders, but the difference between groups did not reach statistical significance (Table 2).

At end of treatment, $\log_{10}$ HBsAg was well correlated with $\log_{10}$ HBeAg ($r = 0.567$, $P = 0.001$) and $\log_{10}$ (serum HBV DNA) ($r = 0.591$, $P = 0.001$), as well as with $\log_{10}$ cccDNA ($r = 0.673$, $P < 0.001$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.531$, $P = 0.006$). Log_{10} HBeAg had significant correlation with $\log_{10}$ (serum HBV DNA) ($r = 0.682$, $P < 0.001$), $\log_{10}$ cccDNA ($r = 0.678$, $P < 0.001$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.753$, $P < 0.001$). Log_{10} (serum HBV DNA) was also significantly correlated with $\log_{10}$ cccDNA ($r = 0.614$, $P = 0.001$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.684$, $P < 0.001$).

Reduction of HBsAg and HBeAg was correlated with reduction of $\log_{10}$ cccDNA ($r = 0.421$, $P = 0.032$, and $r = 0.570$, $P = 0.002$, respectively) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.420$, $P = 0.033$, and $r = 0.535$, $P = 0.005$, respectively), whereas reduction of $\log_{10}$ HBV DNA was not significantly correlated with reduction of $\log_{10}$ cccDNA ($r = 0.365$, $P = 0.066$) and $\log_{10}$ (total intrahepatic HBV DNA) ($r = 0.330$, $P = 0.099$).

Kinetics of serum HBsAg, HBeAg and HBV DNA levels

During treatment and follow up, patients who developed VR showed a consistent decline in serum HBsAg, with a mean decrease of 0.41 ± 0.36, 0.69 ± 0.61,

Table 2 Characteristics of responders and non-responders at end of treatment

	Responder ($n = 10$)	Non-responder ($n = 20$)	P
ALT level, U/L	50.9 ± 36.0	70.0.0 ± 44.7	0.221
Serum HBsAg level, $\log_{10}$ IU/mL	2.59 ± 0.97	3.41 ± 0.64	0.031
Serum HBeAg level, $\log_{10}$ S/CO	0.28 ± 1.08	2.41 ± 0.94	<0.001
Serum HBV DNA level, $\log_{10}$ copies/mL	3.18 ± 1.57	5.59 ± 177	0.001
cccDNA level, $\log_{10}$ copies/genome equivalent	−1.13 ± 1.06	0.66 ± 0.99	<0.001
Intrahepatic HBV DNA level, $\log_{10}$ copies/genome equivalent	−0.87 ± 0.98	0.82 ± 0.92	<0.001
Necroinflammatory score	2.2 ± 1.2	3.1 ± 1.7	0.133
Fibrosis score	0.6 ± 0.5	1.0 ± 0.9	0.135

ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

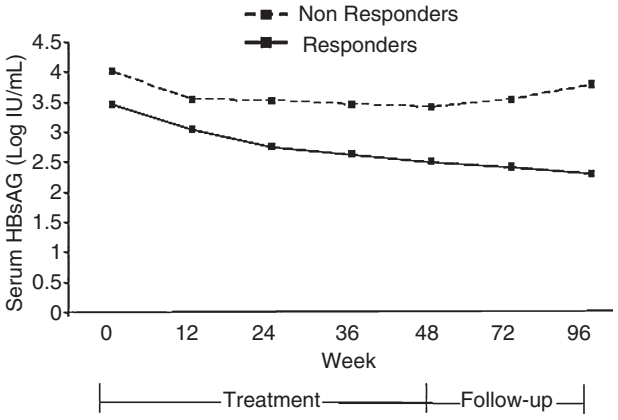

Figure 1 Kinetics of serum hepatitis B surface antigen (HBsAg) during pegylated interferon therapy and follow up in responders (solid line) and non-responders (dashed line).

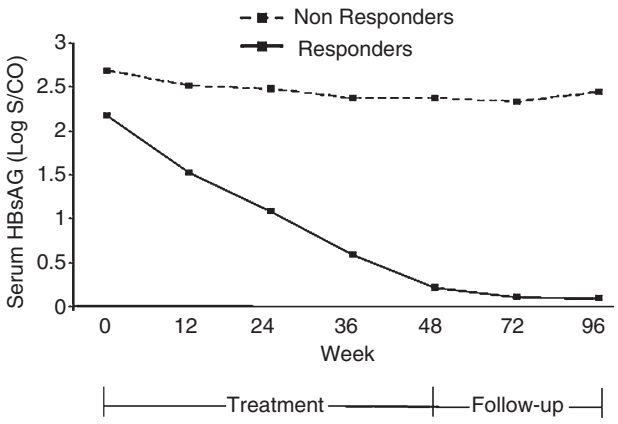

Figure 2 Kinetics of serum hepatitis B e-antigen (HBeAg) during pegylated interferon therapy and follow-up in responders (solid line) and non-responders (dashed line).

0.82 ± 0.67, 0.96 ± 0.75, 1.05 ± 0.84 and 1.15 ± 0.79 $\log_{10}$ IU/mL at weeks 12, 24, 36, 48, 72 and 96, respectively. It should be noted that in this patient group serum HBsAg still decreased after cessation of treatment. Non-responders, however, showed a decrease in HBsAg levels at week 12 which leveled off during weeks 24–48, and had a tendency to rebound after cessation of treatment (Fig. 1).

Patients who developed VR showed a marked decrease in serum HBeAg, with a mean decrease of 0.65 ± 0.81, 1.08 ± 0.98, 1.57 ± 1.00, 1.95 ± 1.32, 2.05 ± 1.22 and 2.07 ± 1.21 $\log_{10}$ S/CO at weeks 12, 24, 36, 48, 72 and 96, respectively. However, serum HBeAg levels did not significantly decrease during treatment in non-responders (Fig. 2).

Patients who developed VR showed a marked decline in serum HBV DNA, with a mean decrease of 1.11 ± 1.30, 1.96 ± 1.55, 2.73 ± 1.69, 2.76 ± 1.69, 3.84 ± 1.65 and 3.92 ± 1.59 $\log_{10}$ copies/mL at weeks 12, 24, 36, 48, 72 and 96, respectively. Patients who did not achieve VR demonstrated slightly decreased HBV DNA levels during therapy which had a tendency to rebound after cessation of treatment (Fig. 3).

Predictors of VR at baseline and during therapy

At baseline, $\log_{10}$ HBeAg provided a prediction of VR comparable to $\log_{10}$ HBsAg but better than $\log_{10}$ HBV DNA. The area under ROC of $\log_{10}$ HBeAg for VR was 0.79 (95% confidence interval [CI], 0.62–0.95; $P = 0.012$), whereas the areas under ROC of $\log_{10}$ HBsAg and $\log_{10}$ HBV DNA were 0.75 (95% CI, 0.57–0.92; $P = 0.029$) and 0.64 (95% CI, 0.44–0.84; $P = 0.210$), respectively.

At week 12 during therapy, $\log_{10}$ HBeAg provided a better prediction of VR than $\log_{10}$ HBsAg and $\log_{10}$ HBV DNA. The area under ROC of $\log_{10}$ HBeAg for VR was 0.83 (95% CI, 0.68–0.98; $P = 0.004$), whereas the areas under ROC of $\log_{10}$ HBsAg and $\log_{10}$ HBV DNA were 0.73 (95% CI, 0.55–0.91; $P = 0.043$) and 0.73 (95% CI, 0.53–0.92; $P = 0.041$), respectively.

Similarly, quantitative HBeAg provided the best prediction of VR at week 24. The area under ROC of $\log_{10}$ HBeAg for VR was 0.91 (95% CI, 0.79–1.01; $P = 0.001$),

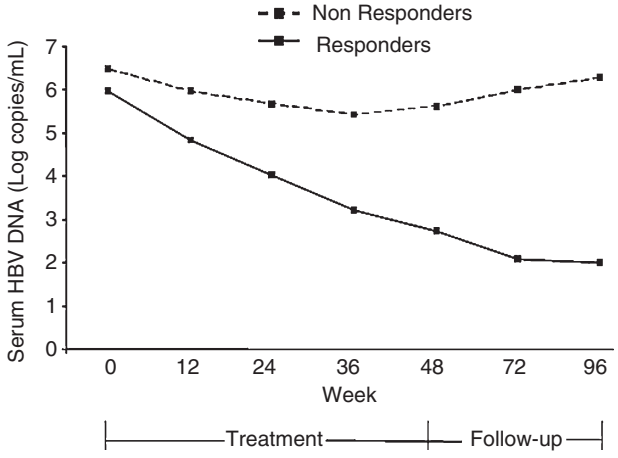

Figure 3 Kinetics of serum hepatitis B virus (HBV) DNA during pegylated interferon therapy and follow up in responders (solid line) and non-responders (dashed line).

Table 3 HBsAg, HBeAg and HBV DNA levels to predict virological response at week 96

	Sensitivity	Specificity	PPV	NPV	Accuracy
Week 12					
Log_{10} HBsAg <3.4 IU/mL	65.0	70.0	52.0	80.0	68.3
Log_{10} HBeAg <2.4 S/CO	75.0	80.0	65.2	86.5	78.3
Log_{10} HBV DNA <5.0 copies/mL	70.0	70.0	53.9	82.4	70.0
Week 24					
Log_{10} HBsAg <3.2 IU/mL	65.0	80.0	61.9	82.1	75.0
Log_{10} HBeAg <2.0 S/CO	85.0	90.0	80.9	92.3	88.3
Log_{10} HBV DNA <4.6 copies/mL	75.0	75.0	57.7	85.3	73.3

HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NPV, negative predictive value; PPV, positive predictive value; S/CO, sample to cut-off ratio.

whereas the areas under ROC of log_{10} HBsAg and log_{10} HBV DNA were 0.78 (95% CI, 0.62–0.95; $P = 0.013$) and 0.80 (95% CI, 0.62–0.98; $P = 0.009$), respectively.

Based on ROC curve analysis, a cut-off point for serum HBsAg, HBeAg and HBV DNA levels considered most accurate for predicting VR was determined. At week 24 of treatment, HBeAg level below 2.0 log_{10} S/CO (100 S/CO) had a sensitivity of 85%, and NPV of 92.3% to predict VR. At the same time point, HBV DNA concentrations below 4.6 log_{10} copies/mL (~40 000 copies/mL) had sensitivity and NPV of 75% and 85.3%, respectively, while the sensitivity and NPV of HBsAg levels below 3.2 log_{10} IU/mL (~1600 IU/mL) were 65% and 82.1%, respectively (Table 3).

HBsAg clearance

In this study, HBsAg clearance occurred in one patient (3.3%), who also achieved VR. The kinetics of serum HBsAg of this patient compared with those who developed VR without HBsAg clearance are shown in Figure 4. The patient who cleared HBsAg had a relatively low pretreatment HBsAg level and a more rapid decline of the protein level during therapy, particularly after the first 12 weeks of PEG-IFN therapy than those who did not clear HBsAg. In the patient who cleared HBsAg, the decrease in serum HBsAg at weeks 12, 24, 36, 48, 72 and 96 was 0.60, 1.58, 1.95, 2.35, 2.70 and 2.70 log_{10} IU/mL, respectively, while the mean decrease in serum HBsAg was 0.41 ± 0.38, 0.60 ± 0.55, 0.70 ± 0.57, 0.81 ± 0.60, 0.87 ± 0.65 and 1.03 ± 0.59 log_{10} IU/mL, respectively, in patients who did not clear HBsAg.

DISCUSSION

ALTHOUGH THE ELIMINATION of HBsAg is the ultimate goal in the management of chronic hepatitis B, it is uncommon in clinical practice, accounting for less than 5% of cases associated with PEG-IFN therapy.[17] In this study, HBsAg clearance was achieved in approximately 3% of patients. Another more realistic end-point in HBeAg-positive patients is HBeAg seroconversion, which has been considered a surrogate marker for long-term therapeutic response and improved clinical outcome of the patients.[17] In recent meta-analysis studies, patients treated with IFN-based therapies who achieved HBeAg seroconversion had significantly decreased HBV-related complications, including the development of cirrhosis and HCC.[18,19] In this study, the rate of HBeAg seroconversion (~33%) was comparable to previous reports involving a larger number of patients treated with PEG-IFN alone or in combination with lamivudine.[20,21]

In current treatment recommendations, monitoring serum HBV DNA levels at baseline and during therapy

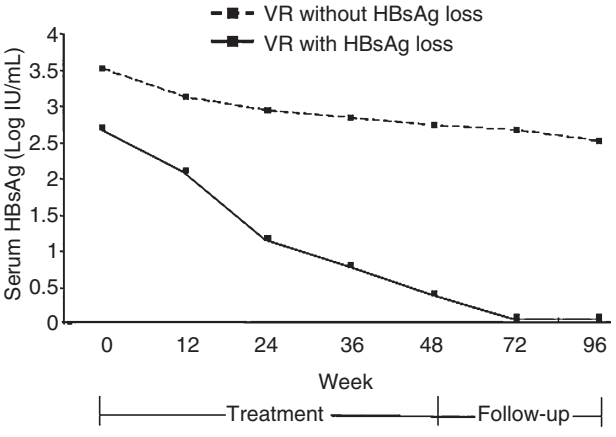

Figure 4 Kinetics of serum hepatitis B surface antigen (HBsAg) during pegylated interferon therapy and follow up in a patient who achieved virological response (VR) and cleared HBsAg (solid line) and those who developed VR without HBsAg clearance (dashed line).

has been practically considered to predict the response to IFN-based therapies.[4] However, measurement of serum HBV DNA level may not always accurately reflect the state of disease in the liver. In recent years, the development of methods for quantification of intrahepatic HBV replicative forms has provided additional molecular markers to predict antiviral treatment response.[22–24] It has recently been shown that cccDNA and total intrahepatic HBV DNA levels, which represent the active replicative and transcriptional form of the virus, are superior to serum HBV DNA as predictors of VR to antiviral therapy in patients receiving either lamivudine monotherapy or combination therapy of lamivudine and PEG-IFN.[25] Nonetheless, a major drawback in measuring cccDNA is the requirement for liver tissue. To avoid this obstacle, the validation of non-invasive surrogate markers such as the quantification of serum HBsAg and HBeAg is required in the clinical setting of antiviral therapy.[26]

In this study, we showed that baseline serum HBV DNA levels did not correlate with the amount of intrahepatic viral DNA and were not associated with VR to PEG-IFN therapy. In contrast, baseline HBsAg and HBeAg levels were significantly lower in responders than in patients who did not show HBeAg seroconversion. These observations are not surprising as both serum HBsAg and HBeAg levels prior to treatment were well correlated with intrahepatic HBV replicative forms. Indeed, low levels of cccDNA and total intrahepatic HBV DNA at baseline represented good predictors of VR, indicating that patients who will most likely benefit from the treatment are those who tend to have lower virus levels in the liver.[25,27] Our findings are in agreement with previous data in that low baseline HBsAg is more reliable than serum HBV DNA levels for predicting good response to PEG-IFN and lamivudine treatment in HBeAg-positive patients.[6] In HBeAg-negative patients, low pretreatment HBsAg level was considered the only significant prognostic predictor of HBsAg seroconversion by multivariate analysis following conventional IFN treatment.[28] Likewise, a recent large study of PEG-IFN-α-2a therapy demonstrated that HBeAg seroconversion was significantly associated with pretreatment HBeAg concentrations; as pretreatment level of HBeAg increased, the rate of subsequent HBeAg seroconversion diminished.[7]

It has been demonstrated that dynamic monitoring of quantitative HBeAg may be useful to predict a response to conventional IFN therapy.[29–31] Our data showed that, among responders, more rapid and consistent decline of HBeAg levels was observed during therapy and follow-up period. We demonstrated that serial measurement of HBeAg levels was superior to HBV DNA and HBsAg levels in predicting the likelihood of subsequent HBeAg seroconversion. At week 24 of treatment, HBeAg levels exceeding 2.0 $\log_{10}$ S/CO (100 S/CO) had an NPV for predicting sustained VR of approximately 92%, which surpassed that obtained by quantitative HBsAg (82%) and HBV DNA (85%) analysis. This high NPV of serum HBeAg at week 24 could allow the selection of patients who should cease therapy to reduce unnecessarily prolonged exposure to costly and unpleasant side-effects of PEG-IFN. In fact, such a "stopping rule" of quantitative HBeAg at week 24 of therapy has been well addressed by recent data using PEG-IFN-α-2a therapy,[7] which may be analogous to the current recommendation for patients with chronic hepatitis C treated with PEG-IFN and ribavirin.

The reason why dynamic monitoring of HBeAg levels offered a better prediction of VR than that of serum HBV DNA levels could be explained by the pattern of HBV DNA decline induced by PEG-IFN. Based on the results of a recent study of PEG-IFN-α-2b monotherapy, a proportion of patients with treatment response had a late decline of HBV DNA up to week 32 of therapy.[32] In our study, 30% of patients with HBeAg seroconversion had a late decline of HBV DNA after week 24 of treatment. Thus, VR in PEG-IFN monotherapy could not be predicted sufficiently on the basis of HBV DNA decline at an early stage of treatment. As a result, the measurement of HBeAg levels as early as week 12 or 24 of PEG-IFN monotherapy could provide a better predictor than HBV DNA monitoring. It should be noted that an early and significantly more pronounced decline of HBV DNA levels was observed in patients who received the combination therapy of lamivudine and PEG-IFN than in those treated with PEG-IFN alone.[32] Consequently, it was shown that an early decrease in HBV DNA could possibly be a predictor of treatment response to the combination therapy.[33]

Unlike HBV DNA, the reductions of HBsAg and HBeAg paralleled with the reduction of cccDNA, suggest that the decline of HBV proteins in serum reflect a diminished cccDNA pool in the liver. Indeed, serum HBsAg and HBeAg are produced by transcription and translation of the surface and precore/core genes of the HBV genome, respectively.[34] These data further confirm that quantitative HBsAg, as well as HBeAg titers, may be useful surrogate markers for cccDNA and may have clinical applicability for predicting VR to PEG-IFN therapy.[6,27] In this study, although monitoring HBsAg levels in the whole study population appeared to be less

indicative for VR than HBeAg levels, the reduction of HBsAg in individuals could predict HBsAg clearance. Previous data have demonstrated that mathematical modeling of HBsAg decline can predict HBsAg clearance for both IFN-based and NA-based therapies.[28] Interestingly, a recent study suggested that a rapid decline in HBsAg levels was associated with a high likelihood of HBsAg clearance in HBeAg-negative patients who had been treated with PEG-IFN.[13] Moreover, an HBsAg level below 10 IU/mL at week 48 and a decline exceeding 1 $\log_{10}$ IU/mL during treatment were significantly associated with HBsAg clearance during follow up in HBeAg-negative patients treated with PEG-IFN.[5] In this study, our data showed that the patient who cleared HBsAg at week 72 exhibited a more rapid decline in serum HBsAg during therapy than those who developed VR without HBsAg clearance. Thus, our results confirmed that a significant decline in HBsAg concentration during therapy might be used to predict HBsAg clearance during the follow-up period. These data also highlight the effects of PEG-IFN therapy in modulating host immune response, resulting in sustained response after treatment and eventual HBsAg clearance.

In conclusion, our data suggest that pretreatment quantitative HBsAg and HBeAg determination may be useful for predicting the response to PEG-IFN therapy. Quantitative measurement of HBeAg during therapy may provide better prediction of treatment response than HBV DNA and HBsAg levels. Also, serial monitoring of HBsAg levels during therapy may help predict HBsAg clearance after cessation of treatment. Further studies on larger sample sizes will be required to confirm these observations.

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