Early Serum HBsAg Drop: A Strong Predictor of Sustained Virological Response to Pegylated Interferon Alfa-2a in HBeAg-Negative Patients

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Pegylated interferon alfa-2a (PEG-IFN) may induce sustained virological response (SVR) in 20% of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients. In addition, loss of hepatitis B surface antigen (HBsAg) is achieved with a 10% yearly rate after treatment cessation in sustained responders. The aim of this study was to assess on-treatment serum HBsAg kinetics to predict SVR in HBeAg-negative patients treated with PEG-IFN. Forty-eight consecutive patients were treated with PEG-IFN (180 μg/week) for 48 weeks. Serum hepatitis B virus (HBV) DNA (COBAS TaqMan) and HBsAg (Abbott Architect HBsAg QT assay) were assessed at baseline, during treatment (weeks 12, 24, and 48), and during follow-up (weeks 72 and 96). SVR was defined as undetectable serum HBV DNA (<70 copies/mL) 24 weeks after treatment cessation. Twenty-five percent of patients achieved SVR. They were not different from those who failed treatment regarding age, sex, ethnicity, HBV genotype, baseline serum HBV DNA and HBsAg levels, or liver histology. During treatment, serum HBsAg levels decreased only in patients who developed SVR, with mean decreases of 0.8 ± 0.5, 1.5 ± 0.6, and 2.1 ± 1.2 $\log_{10}$ IU/mL at weeks 12, 24, and 48, respectively. A decrease of 0.5 and 1 $\log_{10}$ IU/mL in serum HBsAg levels at weeks 12 and 24 of therapy, respectively, had high predictive values of SVR (negative predictive value [NPV] 90%, positive predictive value [PPV] 89% for week 12; NPV 97%, PPV 92% for week 24). HBsAg loss was observed in three patients, all with SVR. *Conclusion:* Early serum HBsAg drop has high predictive values of SVR to PEG-IFN in HBeAg-negative CHB patients. Serum quantitative HBsAg may be a useful tool to optimize the management of PEG-IFN therapy in these patients. (HEPATOLOGY 2009;49:000-000.)

Substantial advances have been made in the treatment of chronic hepatitis B (CHB) in the past decade. Several nucleos(t)ide analogues are currently approved for the treatment of hepatitis B virus (HBV) infection with a high efficacy in suppressing HBV replication. However, a long duration of treatment is needed to maintain viral suppression, and the major question of whether oral therapy can ever be stopped remains unanswered.[1] In parallel with analogues, the American Association for the Study of Liver Diseases practice guidelines have advocated pegylated interferon alfa-2a (PEG-IFN) as a potential first-line therapy in hepatitis B e antigen (HBeAg)-negative patients.[2] The advantages of PEG-IFN therapy include a limited treatment course, a high rate of HBeAg seroconversion (in HBeAg-positive patients), a 20% to 30% rate of sustained virological response (SVR), the potential for hepatitis B surface antigen (HBsAg) loss or seroconversion, and a lack of resistance development.[3] Nonetheless, the use of PEG-IFN currently accounts for no more than 10% of all prescriptions for hepatitis B treatment in the United States and Europe.[4] This low percentage of PEG-IFN therapy may be related to its side effects and the requirement that it should be administered

Abbreviations: ALT, alanine aminotransferase; CHB, chronic hepatitis B; EOT, end of treatment; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IQR, interquartile range; NPV, negative predictive value; PEG-IFN, pegylated interferon alfa-2a; PPV, positive predictive value; SVR, sustained virological response.

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via injection. Another contributing element is the lower antiviral potency of PEG-IFN compared with analogues, and the substantial risk of relapse after cessation of therapy in HBeAg-negative patients. In this regard, serum HBV DNA was undetectable in 63% of patients at the end of PEG-IFN therapy, but in only 19% of patients 6 months after treatment cessation.[5] Interestingly, the durability of this virological response has been recently demonstrated in a study wherein HBV DNA was still undetectable 4 years after the cessation of PEG-IFN therapy in these patients; more important was the occurrence of HBsAg loss with a high steady rate (10% per year) in SVRs.[6] These data highlight the urgent need of predictors that allow the selection of patients who will likely benefit from 1 year of PEG-IFN or, alternatively, will determine whether extensive or indefinite treatment with a nucleos(t)ide analog is likely. Recently, on-treatment serum HBeAg levels were used in HBeAg-positive patients as a quantitative tool to predict SVR to PEG-IFN and showed high negative predictive values (NPVs) at week 12 and 24 of therapy.[7] In HBeAg-negative patients, a recent pilot study including a small number of patients revealed that patients with virological response to PEG-IFN exhibited a significant decrease in serum HBsAg levels during the treatment period in comparison with nonresponders, suggesting that serum HBsAg may also be used as a quantitative tool in this treatment strategy.[8]

The aim of this study was to assess on-treatment serum HBsAg kinetics in HBeAg-negative CHB patients treated with PEG-IFN to predict SVR in the early phase of treatment.

Patients and Methods

Patient Population. Forty-eight consecutive HBeAg-negative patients were evaluated. CHB was documented by the presence of HBsAg in serum for more than 6 months, and by liver biopsy showing histological features of chronic hepatitis compatible with HBV infection. Patients were treated with PEG-IFN at a dose of 180 μg/week for 48 weeks. They were seen every 4 weeks during treatment. Thereafter they were scheduled for follow-up visits every 12 weeks. End of treatment (EOT) response was defined as undetectable serum HBV DNA at the EOT. SVR was defined as undetectable serum HBV DNA 24 weeks after EOT. Relapse was defined as undetectable serum HBV DNA at the EOT and a subsequent detectable serum HBV DNA within the 24 weeks after treatment cessation. Nonresponse was defined as detectable serum HBV DNA at the EOT. All patients gave their informed consent before liver biopsy.

Laboratory Measurements. HBV genotype was determined using the TRUGENE HBV genotyping kit. Serum HBV DNA was measured using the TaqMan polymerase chain reaction assay (COBAS TaqMan, Roche Molecular System [lower limit of detection, 70 copies/mL]) at baseline, during therapy (weeks 12, 24, and 48), and during follow-up (weeks 72 and 96). Serum HBsAg was quantified at the same intervals using the Abbott Architect HBsAg QT assay. Architect HBsAg is a two-step immunoassay based on a chemiluminescent microparticle immunoassay technology that uses microparticles coated with monoclonal anti-HBs for the quantitative determination of HBsAg in serum and plasma. In the first step, sample and anti-HBs–coated paramagnetic microparticles are combined. HBsAg present in the sample binds to the anti-HBs–coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added in the second step. Following another wash cycle, pretrigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units. A direct relationship exists between the amount of HBsAg in the sample and the relative light units detected by the Architect optical system. The concentration of HBsAg in the specimen is determined using a previously generated Architect HBsAg calibration curve (range, 0.05-250 IU/mL). Samples are finally diluted at 1:20 and 1:500 with the Architect HBsAg diluent in order to expand the upper limit of the dynamic range from 250 to 125,000 IU/ml.

Liver Histology. Liver biopsy was obtained for all patients at the start of therapy. Necroinflammation and fibrosis were assessed using the METAVIR score. Necroinflammation activity was graded as A0 (absent), A1 (mild), A2 (moderate), or A3 (severe). Fibrosis stage was graded as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis), and F4 (cirrhosis).

Statistical Analyses. Quantitative variables were expressed as the mean $\pm$ standard deviation or the median with interquartile ranges (IQR), and categorical variables as absolute and relative frequencies. Comparisons between groups of quantitative and qualitative variables were performed using the Mann-Whitney test and the Fisher's exact test, respectively. Comparisons between different groups were performed using the Kruskal-Wallis test. Correlation between serum HBsAg and HBV DNA levels was performed using Spearman rank correlation. The accuracy of serum HBsAg drop to predict SVR was assessed using the receiver operating characteristic curve. All tests were two-sided and used a significance level of 0.05. Data handling and analysis were performed with SPSS software for windows, version 12.0 (SPSS Inc., Chicago, IL).

Table 1. Baseline Characteristics

Characteristic	All Patients (n = 48)	SVR + (n = 12)	SVR − (n = 36)	*P* Value
Age, years	44 (38-53)	45 (42-54)	43 (36-53)	0.2
Sex, % male	83	83	83	1.0
Ethnicity, % Caucasian	67	67	67	1.0
Serum ALT (IU/L)	98 (60-240)	220 (120-390)	90 (54-172)	0.006
HBV genotype (% A, B, C, D, E)	27, 17, 12, 29, 14	41, 17, 8, 25, 8	22, 17, 14, 30, 16	0.2
Serum HBV DNA (log copies/mL)	7.0 (5.5-8.0)	8.0 (6.1-8.8)	6.8 (5.5-8.0)	0.1
Serum HBsAg (log IU/mL)	3.8 (3.2-4.2)	3.9 (2.9-4.3)	3.8 (3.2-4.1)	0.8
Liver necroinflammation, % A2-A3	50	50	50	1.0
Liver fibrosis, % F3-F4	50	50	50	1.0

Data are expressed as the median (IQR) and as percentages.

Results

Baseline Characteristics. Baseline characteristics of the 48 patients are shown in Table 1. Forty patients were male (83%). Thirty-two patients were Caucasian (67%), 10 (21%) were African, and six (12%) were Asian. Median age was 44 years (IQR, 38-53 years). The median value of serum alanine aminotransferase (ALT) was 98 IU/L (IQR, 60-240 IU/L). The distribution of HBV genotype was: A, 27%; B, 17%; C, 12%; D, 29%; and E, 14%. The median value of serum HBV DNA was 7.0 $\log_{10}$ copies/mL (IQR, 5.5-8.0 $\log_{10}$ copies/mL). The median value of serum HBsAg was 3.8 $\log_{10}$ IU/mL (IQR, 3.2-4.2 $\log_{10}$ IU/mL). Liver histology revealed moderate to severe necroinflammation (METAVIR score A2-A3) in 24 patients (50%) and severe fibrosis (METAVIR score F3-F4) in 24 patients (50%). Serological tests for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus were negative in all patients.

Virological Response. Of all 48 patients, 30 (62%) showed an EOT response, and 18 (38%) were nonresponders. Twenty-four weeks after treatment cessation, only 12 patients (25%) achieved SVR, while 18 patients relapsed. Serum HBV DNA kinetics according to treatment response is illustrated in Fig. 1. Patients who developed SVR showed a marked decrease in the first 24 weeks of therapy in comparison with nonresponders (4.1 ± 1.9 versus 2.2 ± 1.7 $\log_{10}$ copies/mL, $P = 0.01$ and 5.1 ± 1.9 versus 2.2 ± 2.3 $\log_{10}$ copies/mL, $P = 0.002$ at weeks 12 and 24 respectively) (Fig. 1A). However, serum HBV DNA profile during the first 24 weeks of therapy was not different in patients who developed SVR compared with relapsers (Fig. 1B), with mean decreases of 4.1 ± 1.9 versus 3.0 ± 1.7 $\log_{10}$ copies/mL ($P = 0.1$) and 5.1 ± 1.9 versus 4.2 ± 1.4 $\log_{10}$ copies/mL ($P = 0.2$) at weeks 12 and 24, respectively. By univariate analyses (Table 1), SVR was associated only with high baseline serum ALT levels. Patients with SVR were similar to those without SVR regarding the remaining baseline characteristics: age, sex, ethnicity, HBV genotype, serum HBV DNA and HBsAg levels, and liver histology.

Serum HBsAg Kinetics. Pretreatment serum HBsAg levels were similar in patients who developed SVR compared with those who did not (3.6 ± 0.8 versus 3.6 ± 0.6 $\log_{10}$ IU/mL [$P = 0.8$]) and correlated significantly with baseline serum HBV DNA levels (Spearman rank correlation 0.45 [$P < 0.001$]). During treatment, patients who developed SVR showed a marked decrease in serum HBsAg, with mean decreases of 0.8 ± 0.5 $\log_{10}$ IU/mL, 1.5 ± 0.6 $\log_{10}$ IU/mL, and 2.1 ± 1.2 $\log_{10}$ IU/mL at weeks 12, 24, and 48, respectively. By contrast, serum HBsAg levels did not decrease during treatment in patients who failed to achieve SVR (Fig. 2), particularly in nonresponders (Fig. 2A). However, relapsers showed a slight later on-treatment decline but also a slow continuing off-treatment decline of serum HBsAg (Fig. 2B).

Predictive Values of Serum HBsAg Kinetics on SVR. At week 12 of PEG-IFN therapy, nine patients showed a decrease of serum HBsAg level ≥ 0.5 $\log_{10}$IU/mL. Among these patients, eight developed SVR. By contrast, 35 of the 39 patients who had a decrease of serum HBsAg level < 0.5 $\log_{10}$IU/mL did not develop SVR. At week 24 of PEG-IFN therapy, 12 patients showed a decrease of serum HBsAg level ≥ 1 $\log_{10}$IU/mL. Among these patients, 11 developed SVR. By contrast, 35 of the 36 patients who had a decrease of serum HBsAg level < 1 $\log_{10}$ IU/mL did not develop SVR. Figure 3 illustrates the predictive values of serum HBsAg drop during the first 24 weeks of therapy on SVR. At week 12, the cutoff of 0.5 $\log_{10}$ IU/mL decrease had a positive predictive value (PPV) of 89% and a NPV of 90% (Fig. 3A). At week 24, the cutoff of 1 $\log_{10}$ IU/mL decrease had a PPV of 92%

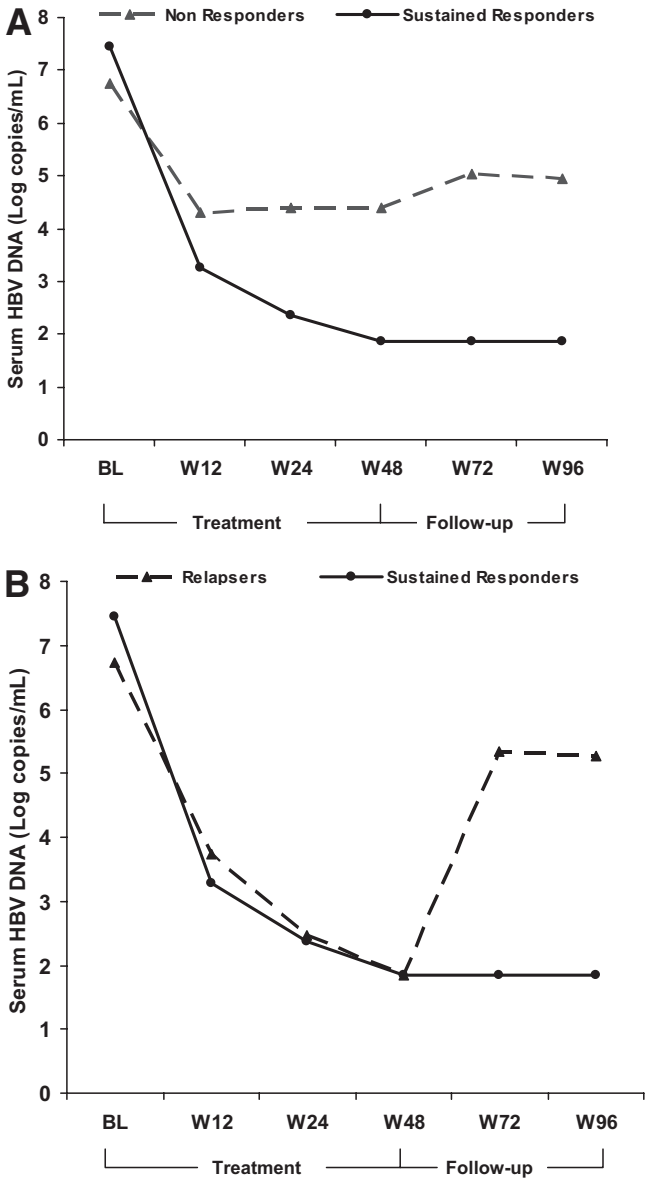

Fig. 1. (A) Kinetics of serum HBV DNA (mean) during the treatment period and follow-up in patients who developed SVR (solid line) and those who did not respond (dashed line). The lower limit of detection of serum HBV DNA is 70 copies/mL (1.85 $\log_{10}$ copies/mL). (B) Kinetics of serum HBV DNA (mean) during the treatment period and follow-up in patients who developed SVR (solid line) and those who exhibited a response at the end of therapy and then relapsed (dashed line). The lower limit of detection of serum HBV DNA is 70 copies/mL (1.85 $\log_{10}$ copies/mL).

and a NPV of 97% (Fig. 3B). The accuracy of the cutoff of 1 $\log_{10}$ IU/mL decrease in serum HBsAg level at week 24 of PEG-IFN therapy to predict SVR was assessed using the receiver operating characteristic curve. The area under the curve was 0.944.

HBsAg Loss. Among the study population (n = 48), HBsAg loss occurred in three patients (6%), all of them have developed SVR. Serum HBsAg kinetics of the three patients compared with the nine who developed SVR without HBsAg loss is illustrated in Fig. 4. The decrease was not different in the first 12 weeks of PEG-IFN therapy (0.7 ± 1.0 versus 0.8 ± 0.4 $\log_{10}$ IU/mL). Thereafter, there was a steeper decline in serum HBsAg during the last 24 months of treatment in patients who lost HBsAg (mean decreases of 2.0 ± 0.9 and 2.9 ± 1.8 $\log_{10}$ IU/mL at weeks 24 and 48 of therapy, respectively) compared with other SVRs who did not (mean decreases of 1.4 ± 0.5 and 2.0 ± 1.0 $\log_{10}$ IU/mL at weeks 24 and 48 of therapy, respectively). It is of note that serum HBsAg still decreased after treatment cessation in patients who did

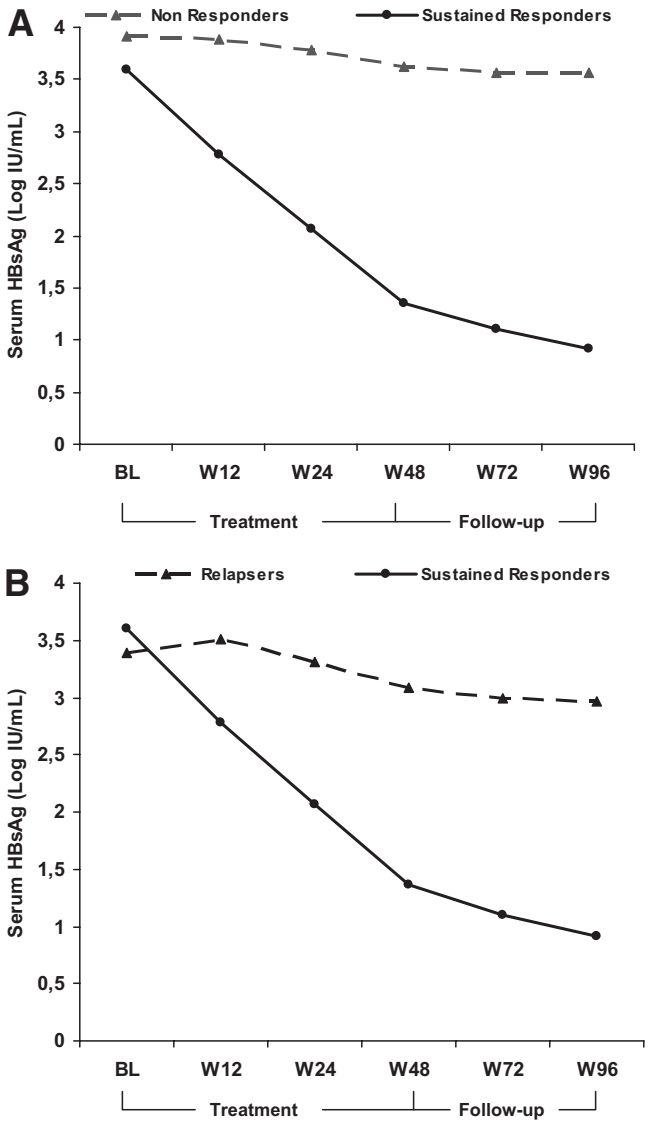

Fig. 2. (A) Kinetics of serum HBsAg (mean) during the treatment period and follow-up in patients who developed SVR (solid line) and those who did not respond (dashed line). (B) Kinetics of serum HBsAg (mean) during the treatment period and follow-up in patients who developed SVR (solid line) and those who exhibited a response at the end of therapy and then relapsed (dashed line).

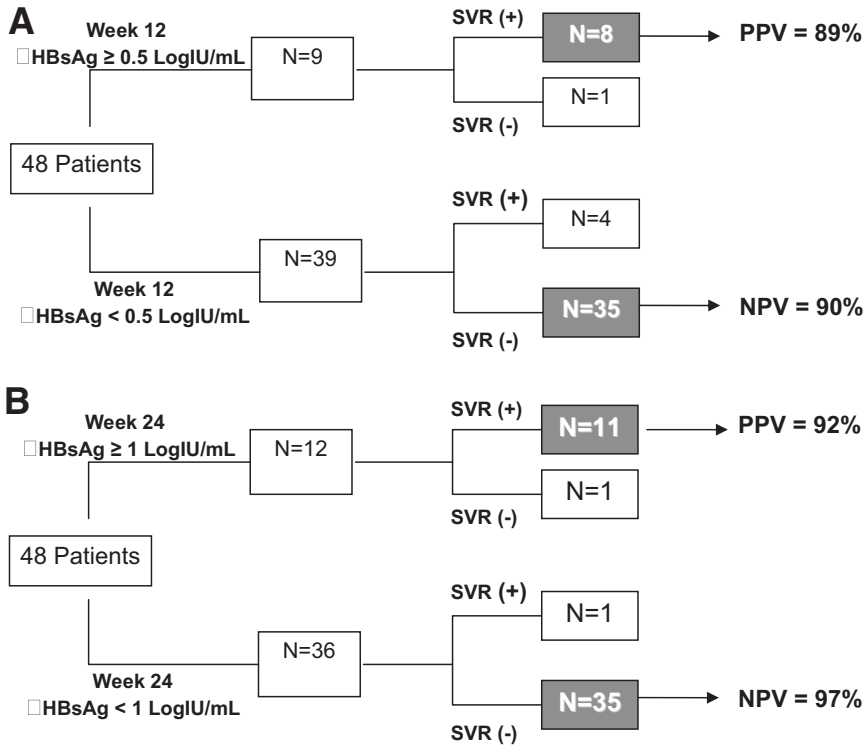

Fig. 3. (A) Predictive values of the cutoff of 0.5 $\log_{10}$ IU/mL decrease in serum HBsAg level at week 12 of PEG-IFN therapy on SVR. (B) Predictive values of the cutoff of 1 $\log_{10}$ IU/mL decrease in serum HBsAg level at week 24 of PEG-IFN therapy on SVR.

not lose HBsAg with mean serum HBsAg levels of 1.3 ± 1.1 and 1.2 ± 1.1 $\log_{10}$ IU/mL 24 and 48 weeks after treatment cessation, respectively, compared with 1.4 ± 1.0 $\log_{10}$ IU/mL at the end of therapy. Figure 5 illustrates the kinetics of serum HBV DNA and HBsAg of the three patients who achieved HBsAg loss separately. In the first patient infected with genotype A, HBsAg and HBV DNA showed parallel kinetics, and loss of HBsAg occurred at the end of therapy when HBV DNA became undetectable (Fig. 5A). In the second and third patients infected with genotype A and D, respectively, HBsAg kinetics showed a delay as compared with HBV DNA, which became undetectable at week 12 of therapy in both patients, while HBsAg loss occurred 24 weeks after treatment cessation in the second patient (Fig. 5B) and 48 weeks after treatment cessation in the third (Fig. 5C). Interestingly, anti-HBs antibodies were slightly positive (12 IU/mL) 48 weeks after treatment cessation in the first patient, whereas they were negative (<10 IU/mL) in the remaining two patients.

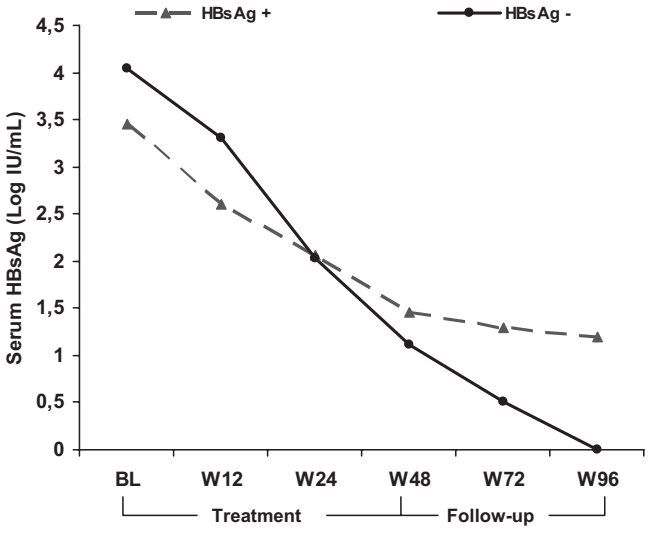

Fig. 4. Kinetics of serum HBsAg (mean) in patients who achieved HBsAg loss (solid line) and those who developed SVR without HBsAg loss (dashed line).

Discussion

A 1-year course of PEG-IFN may induce SVR in 20% of HBeAg-negative patients.[6] This is a major advantage compared with nucleos(t)ide analogues, which need to be continued indefinitely to maintain viral suppression.[9] In this respect, the recurrence of viremia was systematically observed in 33 HBeAg-negative patients who ceased therapy with adefovir after 5 years of treatment following sustained HBV DNA negativity.[10] Given the substantial risk of relapse among patients who exhibited response at the end of PEG-IFN therapy, as shown in our study, it would be of great clinical relevance to identify as early as possible those patients who are likely to develop SVR and, more importantly, relapsers and nonresponders who may benefit from being switched to an alternate treatment strategy. In this study, virological response was defined as

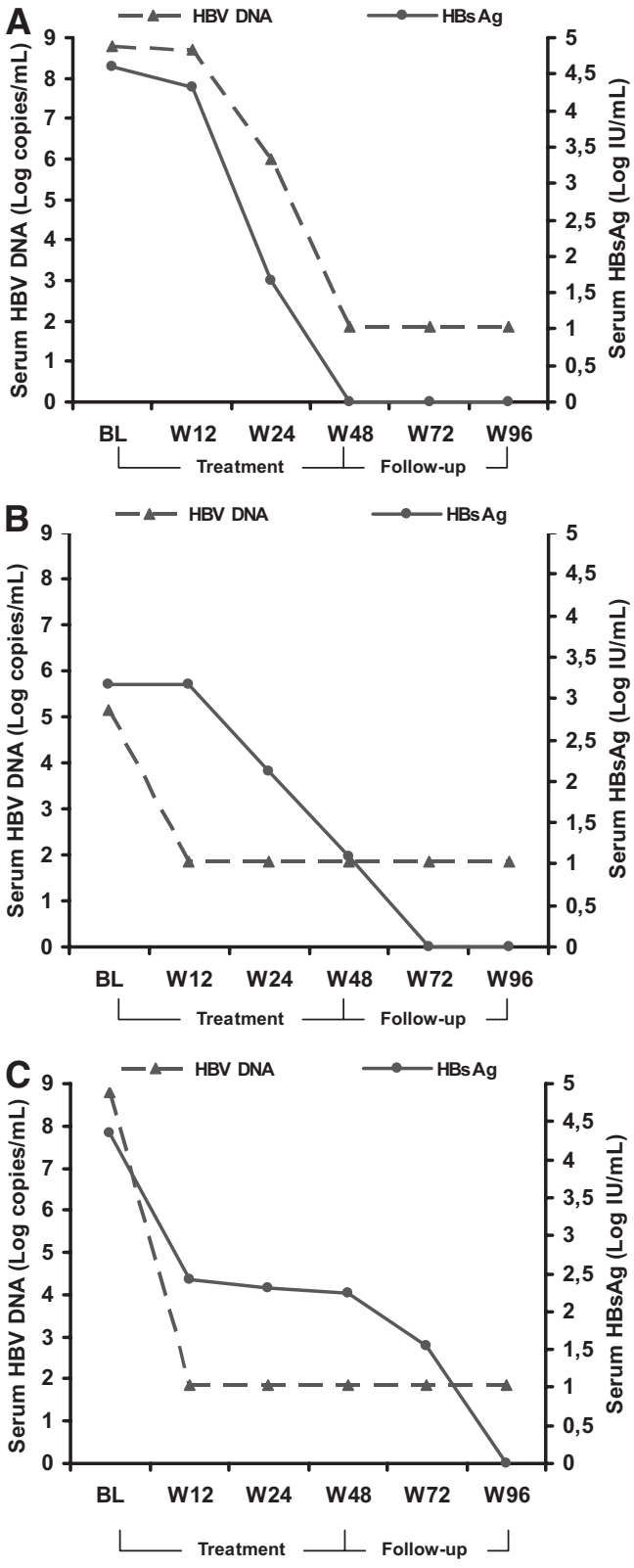

Fig. 5. Kinetics of serum HBsAg (solid line) and serum HBV DNA (dashed line) in the three patients who achieved HBsAg loss. The lower limit of detection of serum HBV DNA is 70 copies/mL (1.85 $\log_{10}$ copies/mL).

undetectable serum HBV DNA rather than <20,000 copies/mL,[5] since it was recently demonstrated[6] that patients with serum HBV DNA <400 copies/mL 24 weeks after treatment cessation (19%) had a durable viral suppression 4 years after treatment cessation (17%) and a high rate of serum HBsAg loss (10% per year). In a previous study of HBeAg-negative patients, several baseline host and viral factors were found to be associated in multivariate analysis with a higher likelihood of virological response to PEG-IFN.[11] Among these factors, considerable attention has been given to the role of HBV genotype, with a lower rate of response observed in genotype D in comparison with other genotypes. In our study, high ALT level was the only baseline factor associated with SVR (Table 1). Although SVRs were nearly twice as likely to have genotype A as those who failed to achieve SVR, there was no significant association between HBV genotype and SVR. This is probably related to a relatively high type II error probability.

Although HBsAg level is determined only qualitatively in routine clinical practice, recent data suggest that quantitative determination of HBsAg level may provide a useful insight into the likelihood of eventual HBsAg seroconversion.[12] These findings are in accordance with the dual antiviral and immunomodulatory effects of PEG-IFN, which result in the suppression of viral replication, but also the clearance of infected hepatocytes. This is supported by the finding that the reduction of serum HBsAg levels parallels the decline of intrahepatic covalently closed circular DNA.[13] In our study, we found high predictive values of on-treatment serum HBsAg kinetics to predict SVR. Interestingly, the high PPV (89%) of the cutoff of 0.5 $\log_{10}$ IU/mL decrease in serum HBsAg at week 12 of therapy will encourage physicians to continue PEG-IFN in patients who fulfill this criteria, especially because the risk of treatment failure is only 10% (NPV, 90%). Moreover, the excellent NPV (97%) of the cutoff of 1 $\log_{10}$ IU/mL decrease in serum HBsAg at week 24 of therapy will allow physicians to stop PEG-IFN and avoid the expense and inconvenience of unnecessary therapy. By analogy with the early virological response in hepatitis C patients treated with PEG-IFN and ribavirin, this early serum HBsAg drop may be used as an early serological response, which allows a change in the paradigm of therapy in HBeAg-negative patients. Interestingly, the kinetics of serum HBV DNA during the first 24 weeks of therapy did not distinguish sustained responders from relapsers (Fig. 1B) who represent 38% of the whole study population, and 60% of those who exhibited an EOT response. It is of note that the potential value of quantification of serum HBsAg during treatment for pre-

dicting response to conventional interferon was suggested previously in HBeAg-positive patients.[14]

Another major finding was the high rate of HBsAg loss, which developed in three of 12 sustained responders. Interestingly, patients who developed SVR without HBsAg loss continued to decrease their serum HBsAg level after treatment cessation. This observation suggests that prolongation of treatment duration may be relevant in patients with steady decline of serum HBsAg, especially if they have good tolerance to treatment. In this respect, the slow continuing decline of serum HBsAg observed in relapsers is perhaps similar to what we see in hepatitis C, where therapy extended to 72 weeks clearly reduces the relapse rate in a subset of patients. It is of note that prolongation of treatment has been tested with conventional interferon[15] and recently with PEG-IFN in a small pilot HBeAg-negative study.[8] Larger studies are therefore needed to validate this concept and to verify if measurement of HBsAg can be useful in determining to the duration of treatment in HBeAg-negative patients with PEG-IFN.

Finally, loss of serum HBsAg did not closely parallel the decline in serum HBV DNA as depicted in Fig. 5. This finding underscores the imperfect correlation between these two parameters during therapy, and emphasizes the added value of serum HBsAg quantification. In this respect, the recovery of serum HBsAg after treatment cessation was clearly slower than the recovery of serum HBV DNA in relapsers.

In conclusion, serum HBsAg seems to be an excellent on-treatment quantitative marker for predicting sustained off-treatment response and identifying in the early phase of PEG-IFN therapy patients who will most likely benefit from this treatment. Further large studies using PEG-IFN with or without potent analogues are warranted to confirm these data on a large scale.

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