Original Article

Prediction of the Response to Pegylated Interferon α -2a in Patients with HBeAg Positive Chronic Hepatitis B through Decline of Serum HBV DNA and Hepatitis B Virus Surface Antigen at Week 4

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Objective To assess on-treatment serum HBsAg and HBV DNA kinetics in HBeAg-positive CHB patients to predict the efficacy of pegylated interferon (PEG-IFN) in early phase of treatment.

Methods Forty-one treatment-naïve HBeAg-positive patients treated with PEG-IFN α 2a at a dose of 180 μ g/week for at least 24 weeks were evaluated. Their treatment response was assessed, including normalization of serum ALT, decline of serum HBV DNA and loss of HBeAg.

Results We found that a decrease of HBV DNA level at the 4th week was positively correlated with the decrease of HBV DNA level at the 12th week and 24th week ($r = 0.8202$, $P < 0.0001$ and $r = 0.6838$, $P < 0.0001$, respectively). We observed that a decrease of HBsAg level at the 4th week was positively correlated with decrease of HBsAg level at the 12th week and 24th week ($r = 0.4868$, $P = 0.0023$ and $r = 0.4251$, $P = 0.0109$, respectively). A decrease of HBsAg level at the 24th week was positively correlated with the decrease of HBV DNA level at the 24th week ($r = 0.5262$, $P = 0.0024$). Serum level of IFN and IFN neutralizing antibody had no relationship with HBV DNA or HBsAg titers kinetics.

Conclusions The decline of serum HBV DNA and hepatitis B surface antigen at the 4th week can be used to predict the response to PEG-IFN α 2a in patients with HBeAg positive chronic hepatitis B.

Key words: Response; Antiviral therapy; Pegylated interferon (PEG-IFN); Hepatitis B virus; HBV DNA; HBsAg

Hepatitis B virus (HBV) infection has become one of the most serious public health threats in recent years. Two billion people worldwide have been infected with HBV, and more than 350 million have become victims of chronic hepatitis B (CHB).¹ Several nucleos(t)ide analogues are currently approved for the treatment of HBV infection with a high efficacy in suppressing HBV replication. However, a long duration of treatment is required to maintain viral suppression, and the major question of whether oral therapy can ever be stopped remains unanswered.² The American Association for the Study of Liver Diseases practice guidelines have advocated pegylated interferon (PEG-IFN) α -2a as a potential first-line therapy in hepatitis B e antigen (HBeAg)-positive patients.³ The advantages of PEG-IFN therapy include a limited treatment course, a higher rate of

HBeAg seroconversion, a 20% to 30% rate of sustained virological response (SVR), the potential for hepatitis B surface antigen (HBsAg) loss or seroconversion, and lack of resistance development. The disadvantages of PEG-IFN therapy include its side effects, lower antiviral potency of PEG-IFN compared with analogues, the substantial risk of relapse after cessation of therapy and need of injection. 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 the 12th week and 24th week of therapy.⁴ In HBeAg-negative CHB patients, a recent pilot study including a small number of patients revealed early serum HBsAg drop has high predictive values of SVR to PEG-IFN.⁵ Plasmacytoid dendritic cells (pDCs) are important contributors in host defence as they produce large amounts of type I IFNs, a critical early step for the innate immune system to control viral replication.⁶ Thus, we also assessed the numbers of pDCs in peripheral blood mononuclear cells (PBMC) of CHB

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patients during PEG-IFN treatment.

This study was aimed to assess on-treatment serum HBsAg and HBV DNA kinetics in HBeAg-positive CHB patients to predict efficacy of PEG-IFN in early phase of treatment. Moreover, relationships were assessed between either IFN (endogenous or not) or neutralizing antibody to PEG-IFN levels and serum HBsAg kinetics or virological response to a first-line treatment with PEG-IFN.

PATIENTS AND METHODS

Patient population

This was a retrospective study of 41 patients with CHB with no prior history of treatment with immunomodulatory or antiviral drugs, who were followed up between July, 2010 and October, 2011 at Huashan Hospital, Fudan University. Forty-one treatment-naïve HBeAg-positive patients were evaluated. CHB was documented by the presence of HBsAg in serum for more than 6 months, and by persistently elevated serum alanine aminotransferase (ALT). Patients were treated with PEG-IFN α -2a at a dose of 180 μ g/week for at least 24 weeks. Thereafter they were scheduled for follow-up visits every 4 weeks. Their treatment response was assessed including normalization of serum ALT, decrease in serum HBV DNA level, and loss of HBeAg. At the year 2000 and 2006 in NIH conferences on management of hepatitis B, it was proposed that responses to antiviral therapy of chronic hepatitis B should be categorized as biochemical response (BR) and virologic response (VR). In IFN treatment, VR is identified as a decrease of more than 2 log₁₀ (100-fold) in serum HBV DNA at first 6 months treatment. Informed consents were obtained from all patients before treatment. Experiments and procedures were in accordance with the Helsinki Declaration of 1975, and approved by the Human Ethics Committee of Shanghai Fudan University.

Measurement of liver function and serological HBV markers

Liver function detection such as serum albumin, total bilirubin (TBil) and ALT were performed by standard methods in a clinical setting. Hepatitis B serological markers were tested using a commercially available radioimmunoassay (Abbott). The level of HBV DNA was quantified using a DNA assay (PG Biotech; with a lower limit of detection of 500 copies/ml).

Flow cytometric analysis

Expression of surface markers was analyzed by flow cytometry using conjugated monoclonal mouse anti-human antibodies: fluorescein isothiocyanate (FITC)

anti-human Lin1, phycoerythrin (PE)-Cy5 anti-human CD23, and APC anti-human HLA-DR (eBiosciences). Flow cytometry analysis was performed on FACSCalibur (BD Biosciences) instruments and analyzed by FlowJo software (Tree Star Inc.).

ELISA

IFN levels and neutralizing antibody to IFN α 2a were measured on stored sera at baseline and the 4th, the 12th and the 24th week, using a commercial quantitative sandwich ELISA kit (Bender Med-Systems Diagnostics, Vienna, Austria) according to the manufacturer's instructions. The absorbance was read at 450 nm by an ELISA plate reader.

Statistical analysis

Quantitative variables were shown as median with ranges, and categorical variables as absolute and relative frequencies. Comparisons between different groups were performed by the Wilcoxon Ranksum test and student's *t* test. Correlation between serum HBsAg and HBV DNA levels was performed through Spearman rank correlation. All tests were two-sided and used a significant level of 0.05. Data handling and analysis were performed with STATA software, version 7.0.

RESULTS

Forty-one treatment-naïve patients with CHB were recruited into the study. Baseline characteristics of them are shown in Table 1. The majority of patients was Asian and infected with genotype B or C HBV. All 41 HBeAg-positive patients were in an immune clearance phase of disease, defined by persistently elevated serum ALT. Serological detection for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus were negative in all patients.

Biochemical response

Total of 19 patients (46.3%) achieved ALT normalization at the 24th week. The median ALT levels at the 12th week and the 24th week were significantly lower compared to baseline ($Z = 5.189$, $P < 0.0001$ and $Z = 5.801$, $P < 0.0001$ at the 12th week and 24th week, respectively, Table 2, Figure 1).

Virological response

Total of 4 patients (9.7%) had undetectable HBV DNA levels at the 24th week. And 23 patients (56.1%) achieved virological response (VR). The median HBV DNA levels at the 4th, the 12th and the 24th week were significantly lower compared to baseline ($Z = 4.620$, $P < 0.0001$; $Z = 5.701$, $P < 0.0001$ and $Z = 6.131$, $P < 0.0001$ at the 4th, 12th and 24th week, respectively,

Table 1. Baseline characteristics of 41 treatment-naïve patients with CHB

Indicators	Values
Ages (years), median/range	31 (19 - 42)
Sex (m/f)	30/11
ALT (U/L), median/range	220 (62 - 812)
HBV DNA ($\log_{10}$ copies/ml), median/range	8.8 (5.0 - 9.9)
HBsAg ($\log_{10}$ IU/ml), median/range	4.1 (2.9 - 5.1)
HBeAg	Positive

Figure 1. Characteristics of 41 treatment-naïve patients with CHB.

Notes: A, the ALT levels (U/L) at the 12th and 24th week were significantly lower compared to the baseline ($P < 0.0001$ and $P < 0.0001$, respectively). B, the HBV DNA levels ($\log_{10}$ copies/ml) at the 4th, 12th and 24th week were significantly lower compared to the baseline ($P < 0.0001$, $P < 0.0001$ and $P < 0.0001$, respectively). Data were not normally distributed and therefore were transformed into $\log_{10}$. C, the HBsAg levels ($\log_{10}$ IU/ml) at the 4th, 12th and 24th week were significantly lower compared to the baseline ($P = 0.0065$, $P = 0.0003$ and $P < 0.0001$, respectively). Data were not normally distributed and therefore were transformed into $\log$. D, IFN- α concentration (pg/ml) at the 4th, 12th and 24th week were significantly higher compared to the baseline ($P < 0.0001$, $P < 0.0001$ and $P < 0.0001$, respectively). E, IFN- α antibodies titers (ng/ml) were not significantly different at each time point. F, PDC/PBMC (%) at the 4th week was significantly higher compared to the baseline ($P = 0.0094$). But PDC/PBMC at the 12th and 24th week were significantly lower compared to the baseline ($P = 0.0358$ and $P = 0.0051$, respectively).

Table 2. Biochemical characteristics of the studied patients

Indicators	Baseline	The 4th week	The 12th week	The 24th week
ALT (U/L)	220 (62 - 812)	175 (41 - 1046)	82 (19 - 419)*	55 (14 - 453)*
HBV DNA (log ₁₀ copies/ml)	8.8 (5.0 - 9.9)	7.5 (3.6 - 9.3)*	6.1 (2.7 - 9.2)*	5.1 (2.7 - 8.8)*
HBsAg (log ₁₀ IU/ml)	4.1 (2.9 - 5.1)	3.8 (2.4 - 5.1)*	3.7 (2.1 - 5.1)*	3.6 (1.3 - 5.1)*
HBeAg positive (n)	41	41	38	35
IFN- α concentration (U/L)	22.9 (3.1 - 45.8)	2734 (9.2 - 4224)*	4025 (269 - 4462)*	3884 (14.5 - 4567)*
IFN- α antibodies titers (U/L)	34.9 (1.2 - 191.9)	57.0 (0 - 747.2)	38.8 (0 - 544.7)	55.9 (0 - 698.9)
PDC/PBMC (%)	0.23 (0.04 - 3.67)	0.43 (0.15 - 3.56)*	0.16 (0 - 0.83)*	0.17 (0.01 - 0.52)*

Notes: The numbers outside and inside of the brackets represent corresponding values of medians and the ranges at baseline, the 4th week, 12th week and 24th week, respectively; * means values compared to the baseline, $P < 0.05$.

Figure 2. Correlations among IFN- α , ALT, HBsAg and HBV DNA levels at different time points.

Notes: A, IFN- α concentration in ALT normal group was significantly higher compared to that in ALT abnormal group at the 24th week ($Z = 2.171$, $P < 0.05$). B, a decrease of HBsAg level at the 4th week was positively correlated with that at the 12th week ($r = 0.4868$, $P = 0.0023$). C, a decrease of HBsAg level at the 4th week was positively correlated with that at the 24th week ($r = 0.4251$, $P = 0.0109$). D, a decrease of HBV DNA level at the 4th week was positively correlated with that at the 12th week ($r = 0.8202$, $P < 0.0001$). E, a decrease of HBV DNA level at the 4th week was positively correlated with that at the 24th week ($r = 0.6838$, $P < 0.0001$). F, a decrease of HBsAg level at the 24th week was positively correlated with the decrease of HBV DNA level at the 24th week ($r = 0.5262$, $P = 0.0024$).

Table 2). A decrease of HBV DNA level at the 4th week was positively correlated with the decrease of HBV DNA level at the 12th week and the 24th week ($r = 0.8202$, $P < 0.0001$ and $r = 0.6838$, $P < 0.0001$, respectively, Figure 2). A decrease of HBV DNA level at the 12th week was positively correlated with the decrease of HBV DNA level at the 24th week ($r = 0.7493$, $P < 0.0001$). The cutoff of $1.2 \log_{10}$ copies/ml decrease (sensitivity 75%, specificity 100%) in serum HBV DNA at the 4th week of therapy will encourage physicians to continue to use PEG-IFN in patients whose serum HBV DNA may decrease more than $2 \log_{10}$ copies/ml at the 24th week of therapy (Figure 3).

Serum HBsAg kinetics

Totally, serum HBsAg levels of 2 patients (4.9%) were undetectable at the 24th week, and 11 patients (26.8%) had a decrease of $1 \log_{10}$ copies/ml and 4 patients (9.8%) had a decrease of $2 \log_{10}$ copies/ml in serum HBsAg levels at the 24th week. The HBsAg levels at the 4th, the 12th and the 24th week were significantly lower compared to the baseline ($t = 2.801$, $P = 0.0065$; $t = 3.773$, $P = 0.0003$ and $Z = 3.882$, $P < 0.0001$ at the 4th, 12th and 24th week, respectively, Table 2). A decrease of HBsAg level at the 4th week was positively correlated with the decrease of HBsAg level at the 12th week and the 24th week ($r = 0.4868$, $P = 0.0023$ and $r = 0.4251$, $P = 0.0109$, respectively, Figure 2). A decrease of HBsAg level at the 12th week was positively correlated with the decrease of HBsAg level at the 24th week ($r = 0.5733$, $P = 0.0001$). A decrease of HBsAg level at the 24th week was positively correlated with the decrease of HBV DNA level at the 24th week ($r = 0.5262$, $P = 0.0024$).

HBeAg loss

Among the studied population ($n = 41$), HBeAg loss

occurred in 3 patients (7.3%) at the 12th week and 6 patients (14.6%) at the 24th week, respectively.

IFN- α concentration

Serum IFN- α concentration at the 4th, 12th and 24th week were significantly higher compared to baseline ($t = 9.481$, $P < 0.0001$; $Z = -6.862$, $P < 0.0001$ and $Z = -6.969$, $P < 0.0001$ at the 4th, 12th and 24th week, respectively). IFN- α concentration at the 12th week and 24th week were significantly higher compared to that at the 4th week ($Z = -4.316$, $P < 0.0001$ and $Z = -3.830$, $P = 0.0001$ at the 12th and 24th week, respectively). IFN- α concentration at the 24th week was not significantly different with that of the 12th week ($Z = 0.367$, $P = 0.7136$, Table 2). IFN- α concentration in ALT normal group was significantly higher compared to that in ALT abnormal group at the 24th week ($Z = 2.171$, $P = 0.0300$). IFN- α concentration in VR group was not significantly different compared to that in non-VR group at the 24th week ($Z = 0.205$, $P = 0.8377$). IFN- α concentrations in patients with a decrease $> 1 \log_{10}$ IU/ml in serum HBsAg showed no significant difference compared to that in patients without HBsAg decrease $> 1 \log_{10}$ IU/ml group at the 24th week ($Z = 1.205$, $P = 0.2282$).

IFN- α antibodies titers

There was no significant difference among each time point (Table 2). IFN- α antibodies titers in ALT normal group was not significantly different compared to that in ALT abnormal group at the 24th week ($Z = -0.565$, $P = 0.5720$). IFN- α antibodies titers in VR group was not significantly different compared to that in non-VR group ($Z = 0.274$, $P = 0.7839$). IFN- α antibodies titers in a decrease over $1 \log_{10}$ IU/ml in serum HBsAg group was not significantly different compared to that in a decrease less than $1 \log_{10}$ IU/ml group at the 24th week

Figure 3. The cutoff value of HBV DNA level.

Notes: The cutoff of $1.2 \log_{10}$ copies/ml decrease (sensitivity 75%, specificity 100%) in serum HBV DNA at the 4th week of therapy will encourage physicians to continue to use PEG-IFN in patients whose serum HBV DNA will decrease more than $2 \log_{10}$ copies/ml at the 24th week of therapy.

Figure 4. PDC/PBMC detected by flow cytometric analysis.

Notes: Expression of surface markers was analyzed by flow cytometry using conjugated monoclonal mouse anti-human antibodies: fluorescein isothiocyanate (FITC) anti-human Lin1, phycoerythrin (PE)-Cy5 anti-human CD23, and APC anti-human HLA-DR (eBio-sciences). Red color points represent PDC-iso, and blue color points represent PBMC of patients, and PDC was counted in Q1.

($Z = 0.880$, $P = 0.3790$). IFN- α antibodies titers was not significantly different between male and female patients ($Z = -0.733$, $P = 0.4635$).

PDCs/PBMC

PDC/PBMC was detected by flow cytometric analysis (Figure 4). PDC/PBMC at the 4th, 12th and 24th week were significantly lower compared to that at the baseline ($Z = -2.596$, $P = 0.0094$; $Z = 2.099$, $P = 0.0358$ and $Z = 2.799$, $P = 0.0051$ at the 4th, 12th and 24th week, respectively, Table 2 and Figure 1).

DISCUSSION

In our study, we found serum IFN- α concentration in ALT normal group was significantly higher compared to that in ALT abnormal group at the 24th week, but it had no relationship with HBV DNA or HBsAg titer kinetics. PDC/PBMC at the 4th, 12th and 24th week were significantly lower compared to that at the baseline, thus serum IFN- α concentration did not seem as endogenous for PDC/PBMC continues decrease in the treatment, and was likely to be affected by PEG-IFN. IFN- α antibodies seems not very important in PEG-IFN treatment and not as its role in interferon treatment.⁷⁻⁹ It had no relationship with ALT normalization, HBV DNA or HBsAg titer kinetics, or IFN- α concentration.

Some scholars have found that a decrease $< 60\%$ of baseline levels of HBsAg at the 12th week was considered as an independent predictive factor for HBeAg seroconversion (OR = 45.7, $P < 0.05$) at the 96th week. Serum HBsAg levels may be helpful for predicting the response to PEG-IFN therapy in

HBeAg-positive chronic hepatitis B patients.¹⁰ And a quantitative serum HBsAg level at the 12th week of therapy can be used for the early prediction of sustained response to PEG-IFN therapy in HBeAg-negative CHB patients infected with genotype B or C.¹¹ Moreover, the correlation between quantitative HBsAg titer, and serum and intrahepatic markers of HBV replication differs between patients with HBeAg-positive and HBeAg-negative CHB. These findings suggested that quantitative HBsAg titer can be taken as a clinical biomarker.¹² Recent data suggested that quantitative determination of HBsAg level may provide a useful insight into the likelihood of eventual HBsAg seroconversion.¹³ 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.¹⁴ In our study, it would be of great clinical relevance to identify as early as possible those patients who are likely to develop EVR, and more importantly, relapsers and nonresponders who may be benefited from being switched to an alternate treatment strategy.

In lamivudine-treated patients, early suppression of viral load was linked to greater rates of HBeAg seroconversion, even when advanced liver disease was present.¹⁵ In addition, it was demonstrated that a drop in viral load to HBV DNA levels below 2000 IU/ml at the 4th week could predict an ideal outcome, defined as combined virological and biochemical response at 5th year.¹⁶ However, in a large randomized multicenter

study of PEG-IFN α -2b with or without LAM, a $1\log_{10}$ drop in serum HBV DNA levels at the 32th week of PEG-IFN α -2b monotherapy was predictive of HBeAg loss in genotype A patients only. Earlier predictions were not associated with sustained response in that study.¹⁷ One model strongly supports individual decision making on treatment (dis)continuation in patients with HBeAg positive chronic hepatitis B. It is recommended that PEG-IFN treatment is stopped at the 24th week if HBV DNA declined $< 2 \log_{10}$.¹⁸ However, we found that the decrease of HBV DNA level at the 4th week was positively correlated with the decrease of HBV DNA level at the 12th and 24th week ($r = 0.8202$, $P < 0.0001$ and $r = 0.6838$, $P < 0.0001$, respectively). During hepatitis C treatment, a rapid virological response (RVR), defined as undetectable HCV RNA at the 4th week of treatment, using a sensitive detection with a lower limit detection of 50 IU/ml, predicts a high likelihood of achieving an SVR. An early virological response (EVR) is defined as a $> 2 \log_{10}$ reduction or complete absence of serum HCV RNA at the 12th week of therapy compared with the baseline level. Failure to achieve an EVR is the most accurate predictor of not achieving an SVR.¹⁹ In our study, we found viral kinetics monitoring is thus useful for predicting whether or not an EVR is likely to happen.

In our study, we also found high predictive values of on-treatment serum HBV DNA kinetics to predict EVR. The cutoff of $1.2 \log_{10}$ copies/ml decrease (sensitivity 75%, specificity 100%) in serum HBV DNA at the 4th week of therapy will encourage physicians to continue PEG-IFN in patients whose serum HBV DNA will decrease more than $2 \log_{10}$ copies/ml at the 24th week of therapy. By analogy with the early virological response in hepatitis C patients treated with PEG-IFN and ribavirin, this early serum HBV DNA kinetics may be used as an early serological response, which allows a change in the paradigm of therapy in HBeAg-positive patients. It is of note that the potential value of serum HBV DNA kinetics at the 4th week during treatment for predicting response to PEG-IFN was suggested in HBeAg-positive patients.

In conclusion, serum HBsAg and HBV DNA level at the 4th week seems to be an excellent on-treatment quantitative marker for predicting EVR and identifying who will most likely benefit from PEG-IFN treatment. Further studies using PEG-IFN with or without potent analogues are warranted to confirm these data on a large scale.

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REFERENCES

1. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004;11:97-107.
2. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007;45:1056-1075.
3. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007;45:507-509.
4. Fried MW, Piratvisuth T, Lau GK, Marcellin P, Chow WC, Cooksley G, et al. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. *Hepatology* 2008;47:428-434.
5. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. *Hepatology* 2009;49:1151-1157.
6. Stetson DB, Medzhitov R. Type I interferons in host defense. *Immunity* 2006;25:373-381.
7. Qureshi H, Arif A, Ahmed W, Alam SE, Mujeeb SA, Haq Nu, et al. Role of interferon antibody in predicting the response to interferon therapy in HCV patients. *J Pak Med Assoc* 2007;57:581-583.
8. Halfon P, Prusat S, Bourlire M, Bronowicki JP, Trimoulet P, Benhamou Y, et al. Neutralizing antibodies to interferon- α and circulating interferon in patients with chronic hepatitis C non-responding to pegylated interferon plus ribavirin re-treated by pegylated interferon- α -2a and ribavirin. *J Med Virol* 2010;82:2027-2031.
9. Boo I, Fischer AE, Johnson D, Chin R, Giourouki M, Bharadwaj M, et al. Neutralizing antibodies in patients with chronic hepatitis C infection treated with (Peg)-interferon/ribavirin. *J Clin Virol* 2007;39:288-294.
10. Song EY, Shin Y, Roh EY, Sue S, Park MH, Kim BH, et al. Serum HBsAg levels during peginterferon α -2a treatment with or without thymosin α -1 in HBeAg-positive chronic hepatitis B patients. *J Med Virol* 2011;83:88-94.
11. Peng CY, Lai HC, Li YF, Su WP, Chuang PH, Kao JT. Early serum HBsAg level as a strong predictor of sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2012;35:458-468.
12. Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, et al. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 2010;51:1933-1944.
13. Manesis EK, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. Prediction of treatment-related HBsAg loss in HBeAg-negative chronic hepatitis B: a clue from serum HBsAg levels. *Antivir Ther* 2007;12:73-82.
14. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004;126:1750-1758.
15. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004;351:1521-1531.
16. Yuen MF, Fong DY, Wong DK, Yuen JC, Fung J, Lai CL. Hepatitis B virus DNA levels at week 4 of lamivudine treatment predict the 5-year ideal response. *Hepatology* 2007;46:1695-1703.
17. ter Borg MJ, van Zonneveld M, Zeuzem S, Senturk H, Akarca US, Simon C, et al. Patterns of viral decline during PEG-interferon

-
- alpha-2b therapy in HBeAg-positive chronic hepatitis B: relation to treatment response. *Hepatology* 2006;44:721-727.
18. Hansen BE, Buster EH, Steyerberg EW, Lesaffre E, Janssen HL. Prediction of the response to peg-interferon-alfa in patients with HBeAg positive chronic hepatitis B using decline of HBV DNA during treatment. *J Med Virol* 2010;82:1135-1142.
19. Ghany MG, Strader DB, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-1374.