Original Article

Prediction of HBsAg Loss by Quantitative HBsAg Kinetics during Long-Term Treatment with Nucleos(t)ide Analogues

Sombutsook P
Leerapun A
Praisontarangkul O
Thongsawat S
Chitapanarux T
Pisespongsa P
Kijdamrongthum P

ABSTRACT

Background: Quantitative HBsAg has been regarded as helpful in monitoring and predicting treatment outcome in chronic hepatitis B. The decline of HBsAg is more pronounced in patients receiving Peg-IFN than in those receiving nucleos(t)ide analogues (NUCs). In patients receiving NUCs, the clearance of HBsAg seems very rare especially in Asian populations with genotype B or C infection.

Methods: We retrospectively evaluated different qHBsAg kinetics in 200 patients receiving a nucleos(t)ide analogue for chronic hepatitis B infection with persistently undetectable HBV DNA. HBsAg was quantified at baseline and during antiviral therapy (weeks 24, 48, 96) in HBeAg positive patients (n=84) and HBeAg negative patients (n=116).

Results: For HBeAg positive patients, the reduction of HBsAg in patients with entecavir therapy was pronounced than in those with lamivudine, telbivudine, or lamivudine plus tenofovir therapy (mean decline 0.82 versus 0.72, 0.35 and 0.49 log IU/mL at week 96 respectively, p= 0.41). In patients who had HBeAg seroconversion, the mean declines in HBsAg levels during 96 weeks with entecavir, telbivudine, and lamivudine plus tenofovir therapy were significantly more than in those who had no HBeAg seroconversion (1.31, 0.46 and 0.75 log IU/mL, p=0.015, 0.025 and 0.013, respectively), with the exception of patients treated with lamivudine alone (0.94 log IU/mL, p=0.39). During the first year of treatment, two patterns of HBsAg decline were observed: a rapid decline (≥ 0.5 log IU/mL) and a slow decline (<0.5 log IU/mL). One of 3 patients in the lamivudine group and 2 of 6 patients in the entecavir group with rapid HBsAg decline achieved HBsAg loss during the 96 weeks. For HBeAg negative patients, the mean HBsAg declines during 96 weeks of treatment with lamivudine, entecavir, telbivudine, or lamivudine plus tenofovir were not significantly different (p=0.46). However, all 3 patients with lamivudine therapy and 1 of 3 patients with telbivudine therapy who had a rapid HBsAg decline ultimately achieved HBsAg loss.

Conclusion: In clinical practice, HBsAg quantification and a rapid decline in serum HBsAg level during the first year can be used to predict HBsAg loss in patients who have effective suppression of viral replication during nucleos(t)ide analogue therapy.

Key words : Chronic hepatitis B, HBsAg quantification, nucleos(t)ide analogues

[Thai J Gastroenterol 2015; 16(1):16-22.]

Gastroenterology Unit, Faculty of Medicine, Chiangmai University, Bangkok, Thailand.
Address for Correspondence: *Pises Pisespongsa, M.D., Gastroenterology Unit, Faculty of Medicine, Chiangmai University, Bangkok, Thailand.*

INTRODUCTION

Chronic hepatitis B (CHB) can be controlled in most patients with the currently available treatment options, but complete eradication of the hepatitis B virus (HBV) is rarely achieved. HBV covalently closed circular DNA (cccDNA) plays a major role in viral persistence and its clearance is thought to be the limiting factor in eliminating the infection[1]. Serum HBsAg titer has been shown to correlate with levels of both intrahepatic HBV-DNA and covalent closed circular DNA (cccDNA)[2]. Recently, HBsAg serum measurement (qHBsAg) has been applied to the therapeutic monitoring for its correlation with the antiviral response in patients undergoing PEG-IFN treatment[3,4]. Some athors have reported a significant association between on-treatment decline in serum HBsAg and HBsAg clearance after PEG-IFN treatment. Moreover, serum on-treatment HBsAg kinetics showed a high predictive value for the sustained viral response in these patients[3,4].

The impact of long-term treatment with nucleic acid (NAs) on qHBsAg is still poorly understood. The rate of qHBsAg decline is different between PEG-IFN and NAs therapy, whereas significant qHBsAg decreases have been shown to correlate with interferon administration. Quantitative HBsAg levels during NA treatment showed little and no significant variations from baseline, even when HBV-DNA was undetectable[5]. Therefore, the loss of HBsAg is a difficult endpoint to achieve especially in HBeAg negative patients. Manesis et al. showed an estimated time of HBsAg loss with lamivudine (LAM) administration of 10.6 years (median)[5]. During treatment with nucleos(t)ide analogue, different qHBsAg kinetics were reported for HBeAg(+) and HBeAg(-) patients. Changes of qHBsAg varied, but significant decreases were demonstrated only in HBeAg(+) patients, whereas in HBeAg(-) patients no significant differences between the levels of qHBsAg at baseline and after 2-3 years of treatment have been found[6,7].

The aim of this study was to retrospectively evaluate the qHBsAg kinetics during treatment with NAs in a study group of HBeAg positive and negative patients infected with HBV, in whom HBV-DNA was persistently negative under nucleos(t)ide analogue administration for at least 2 years. A specific aim was to predict the expected HBsAg loss under NAs therapy.

PATIENTS AND METHODS

Patient population

Two hundred out of 500 HBeAg-positive and HBeAg-negative treatment-naive chronic hepatitis B (CHB) patients who received nucleos(t)ide analogue therapy at Maharaj Nakorn Chiangmai Hospital between January 2007 and December 2010 were enrolled. Inclusion criteria were as following; HBeAg positive and negative, treatment with NAs, HBV-DNA persistently undetectable, and at least 2 years of antiviral treatment. Exclusion criteria were as following; previous treatment with IFN or PEG-IFN, co-infection with HIV or HCV, underlying malignancy, less than 2 years of NA administration.

Laboratory tests

Patients attended the outpatient clinic at least every 12 weeks for routine physical examination and laboratory assessment. Serum alanine aminotransferase (ALT) levels were measured using an automated technique. Determination of HBeAg and antibody against HBeAg (anti-HBe) status was performed using a commercially available enzyme immunoassay. Serum HBsAg was quantified at baseline and during antiviral therapy (weeks 24, 48, 96) using the ARCHITECT HBsAg assay. Serum HBV DNA levels were measured using commercial TaqMan polymerase chain reaction (PCR) assay (Roche Molecular Systems; lower limit of detection 34 copies/mL).

Study endpoints

The aim of our study was (i) to assess on-treatment serum HBsAg kinetics in hepatitis B e antigen (HBeAg) positive and HBeAg-negative CHB patients treated with nucleos(t)ide analog, and (ii) to assay the potential use of HBsAg monitoring to identify a subset of patients with effective on-treatment suppression of HBV replication who were likely to achieve HBsAg clearance during long-term NAs therapy.

Statistical analysis

For descriptive statistics, continuous variables were summarized as mean. Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro-Wilk test and categorical data were compared using a Mann-Whitney or Kruskal-Wallis statistical test. To investigate con-

tinuous data, a Spearman Rank correlation was used. The association was calculated using the X^2-test. Two sample t-tests or analysis of variance were used to compare continuous endpoints between any two subgroups. Statistical analyses were conducted by SPSS software package version 17.0. The estimated time to HBsAg loss was evaluated through a linear equation calculated by interpolating median logarithmic decline over time for each single drug.

RESULTS

A total of 200 HBV-infected patients were included in the study. The HBeAg-positive population consisted of 15 patients treated with lamivudine (LAM), 19 patients with entecavir (ETV), 29 patients with telbivudine (LdT) and 21 patients with lamivudine plus tenofovir (LAM plus TDF). The HBeAg-negative population consisted of 33 patients treated with lamivudine, 31 patients with entecavir, 33 patients with telbivudine and 19 patients with lamivudine plus tenofovir. Baseline characteristics are presented in Table 1 and Table 2. HBeAg-positive patients significantly younger (42 vs 50 years, $p < 0.001$) and had higher baseline serum HBV DNA and HBsAg levels compared with HBeAg-negative patients (5.6 vs 4.9 log IU/mL for HBV DNA and 3.5 vs 3.1 log IU/mL for HBsAg, $p= 0.36$ and 0.02 respectively), while median ALT levels were similar (98 vs. 85 IU/mL, $p = 0.44$). However, HBeAg-negative patients were more often cirrhotic than HBeAg-positive patients (13 vs 36, $p= 0.01$). By univariate analysis, the following baseline factors were significantly associated with HBsAg loss: HBsAg level < 1000 IU/mL, and baseline DNA (log IU/mL). After correction of the model for each of these factors in a multivariate analysis, only baseline HBsAg level < 1,000 IU/mL was associated with HBsAg loss ($p = 0.014$).

HBeAg-positive patients

We noted that the mean HBsAg decline from baseline after the 96 weeks of treatment was equal to

Table 1. Baseline characteristics of HBeAg positive patients.

Characteristics	All patients	Lamivudine	Entecavir	Telbivudine	Lamivudine plus tenofovir
Number of patients	84	15	19	29	21
Mean age (yr)	42	40	41	45	39
Male gender, n (%)	52 (61.9)	6 (40)	12 (63.1)	20 (68.9)	14 (66.6)
Baseline HBsAg < 1,000 IU/mL, n (%)	16 (19)	4 (26.7)	4 (21.1)	5 (17.2)	3 (14.3)
Mean qHBsAg (log IU/mL)	3.5	3.6	3.4	3.3	3.7
Mean HBV DNA (log IU/mL)	5.4	5.6	5.86	5.35	4.6
ALT (IU/mL) median [IQR]; (range)	98 (12-632)	88 (21-364)	125 (17-632)	102 (16-370)	37 (12-82)
Cirrhosis, n (%)	13 (15.4)	5 (33.3)	2 (11.7)	5 (17.2)	1 (4.7)

Table 2. Baseline characteristics of HBeAg negative patients.

Characteristics	All patients	Lamivudine	Entecavir	Telbivudine	Lamivudine plus Tenofovir
Number of patients	116	33	31	33	19
Mean age (yr)	50 (31-75)	48 (31-71)	50 (35-70)	53 (36-75)	49 (36-71)
Male gender, n (%)	84 (72.4)	24 (72.7)	25 (80.6)	21 (63.6)	14 (73.6)
Baseline HBsAg < 1,000 IU/mL, n (%)	46 (39.7)	15 (45.5)	12 (38.7)	9 (27.3)	10 (52.6)
Mean qHBsAg (log IU/mL)	3.1	2.9	3.1	3.3	3.2
Mean HBV DNA (log IU/mL) mean	4.9	4.2	5.4	5.0	4.8
ALT (IU/mL) median [IQR]; (range)	85 (14-950)	101 (21-656)	107 (17-850)	58 (14-190)	68 (14-240)
Cirrhosis, n (%)	36 (31)	13 (39)	7 (22.5)	11 (33.5)	5 (26.3)

Table 3. Quantitative HBsAg decline, after 1 and 2 years, during therapy with NAs.

Treatment	HBeAg positive (mean log IU/mL)		HBeAg negative (mean log IU/mL)	
	First year	Second year	First year	Second year
Lamivudine	0.65	0.72	0.24	0.52
Entecavir	0.49	0.82	0.10	0.21
Telbivudine	0.19	0.35	0.16	0.31
Lamivudine plus Tenofovir	0.24	0.49	0.20	0.28
p- value	0.48	0.41	0.31	0.46

0.72 log IU/mL with LAM, 0.35 with LdT, 0.82 with ETV, 0.49 with LAM plus TDF (Table 3). But the mean HBsAg decline was not significantly different among the treatment groups (p= 0.41). The majority of patients (52 [61.9%] of 84) in the study cohort experienced HBeAg loss by year 2 [10/15 (66%) in the lamivudine group, 10/19 (52.6%) in the entecavir group, 20/29 (68.9%) in the telbivudine group, and 12/21 (57.1%) in the lamivudine plus tenofovir group]. In patients who had HBeAg seroconversion, the mean decline of HBsAg level during 96 weeks with entecavir, telbivudine, lamivudine and tenofovir therapy was significantly greater than in those who had no HBeAg seroconversion (1.31, 0.46 and 0.75 log IU/mL, p= 0.015, 0.025, 0.013, respectively) but not significantly different in patients treated with lamivudine (0.94 log IU/mL, p=0.39). However, the magnitude of HBsAg decline in the patients who achieved HBeAg loss was not associated with the treatment regimen (p= 0.42). During the first year of treatment, two patterns of HBsAg decline were observed: a rapid decline (≥ 0.5 log IU/mL) and a slow decline (<0.5 log IU/mL). There were 3 HBeAg positive patients (3.5%) who achieved HBsAg loss. One of 3 patients with lamivudine and 2 of 6 patients with entecavir therapy who exhibited a rapid HBsAg decline achieved HBsAg loss during the 96 weeks of study. In the meantime, no patients with a slow HBsAg decline had HBsAg loss. No association was observed between a rapid, a slow HBsAg decline with ALT normalization and HBeAg seroconversion in HBeAg positive patients.

HBeAg negative patients

We noted that a mean HBsAg decline from baseline after the 96 weeks of treatment was equal to 0.52 log IU/mL with LAM, 0.31 with LdT, 0.21 with ETV, 0.38 with LAM plus TDF (Table 3). But the mean HBsAg decline was not significantly different among the treatment groups (p= 0.46). When comparing the mean HBsAg decline (log IU/mL) between HBeAg positive and HBeAg negative patients, we found that the mean HBsAg decline was not statistically different in each treatment group, except in the entecavir group (0.82 in HBeAg positive vs 0.21 in HBeAg negative, $p < 0.001$, Table 3). There were 4 HBeAg negative patients (3.4%) who achieved HBsAg loss.

All 3 patients in the lamivudine and 1 of 3 patients in the telbivudine therapy with rapid HBsAg decline achieved HBsAg loss during 96 weeks. No patients with slow HBsAg decline had HBsAg loss. No association was noted between a rapid, a slow HBsAg decline and ALT normalization. Univariate analysis showed that the HBsAg loss was related to baseline HBsAg level < 1,000 IU/mL and baseline HBV level but there were no relation between baseline parameter and HBsAg loss in multivariate analysis.

Discussion

The decline of HBsAg during NUCs therapy is less pronounced than that during Peg-IFN therapy[8]. The reason for the slow decline of HBsAg in NUCs treatment is probably because NUCs inhibit only the reverse transcription of the pregenomic RNA but do not target the cccDNA directly. Thus, changes at transcriptional levels, particularly in the HBsAg secretory pathway, are not expected. On the other hand, IFN has both direct antiviral and immune mediated effects. It is likely that the immune modulation by interferon leads to a more dramatic decline in HBsAg production and secretion. In patients receiving NUCs, the decline of HBsAg appears more apparent in HBeAg-positive pa-

tients than in HBeAg-negative patients[9]. Our study showed that the mean HBsAg decline after 96 weeks of treatment with NUCs was not different between HBeAg-positive and HBeAg-negative patients, except in those treated with entecavir (mean HBsAg decline 0.82 log IU/mL in HBeAg(+) vs 0.21 log IU/mL in HBeAg(-), $p< 0.001$). In Wursthorn et al. analyzed quantitative HBsAg in 162 HBeAg positive patients treated with telbivudine for at least 3 years[10]. All patients maintained HBV DNA <60 IU/mL after two years of therapy. Nine patients (6%) developed HBsAg loss through the follow-up of 3 years. A rapid HBsAg decline of more than 1 log after 1 year of treatment was

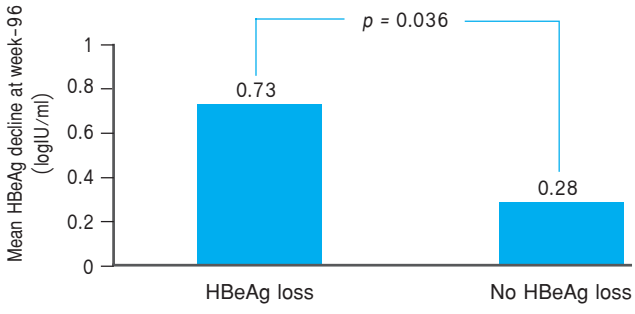

Figure 1. Degree of HBsAg decline at week-96 of NAs therapy for HBeAg-positive patients according to the achievement of HBeAg loss after 96 weeks of treatment.

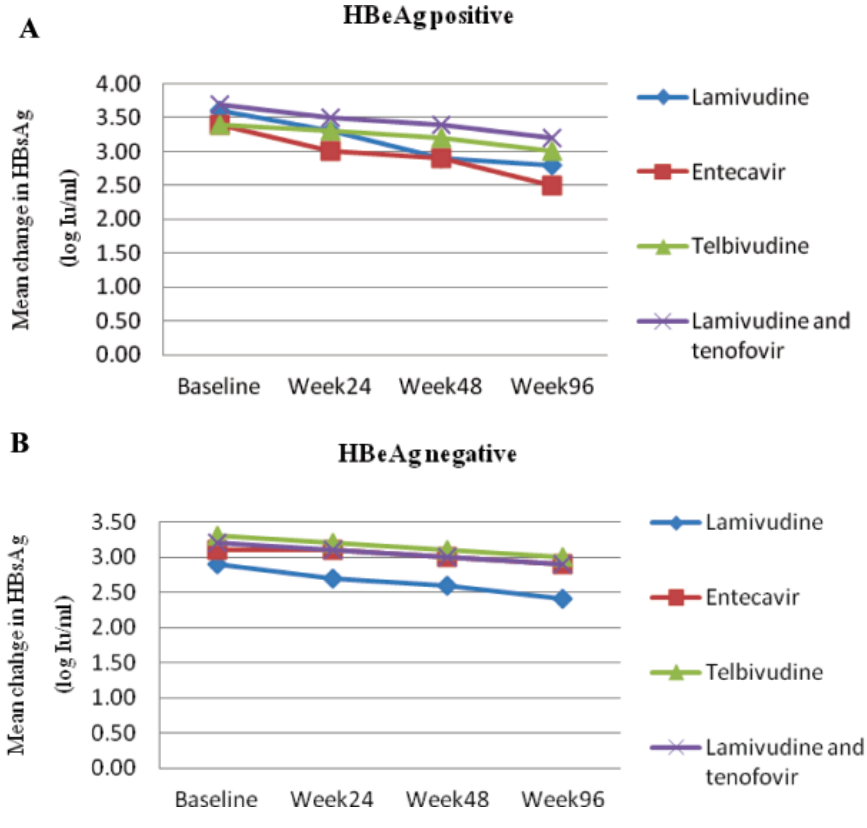

Figure 2. Mean change compared to baseline for HBsAg in HBeAg-positive (A) and HBeAg-negative (B) patients.

Table 4. HBsAg loss in total population at 96 weeks of NUCs therapy according to HBsAg decline at week 48.

	Lamivudine		Entecavir		Telbivudine		Lamivudine and tenofovir	
Decline group	All patients	HBsAg loss	All patients	HBsAg loss	All patients	HBsAg loss	All patients	HBsAg loss
Rapid*	6	4 (66.7%)	6	2 (33.3%)	9	1 (11.1%)	5	0 (0%)
Slow*	42	0 (0%)	44	0 (0%)	53	0 (0%)	35	0 (0%)
p- value	$p < 0.001$		$p = 0.012$		$p = 0.145$		N/A	

[* Rapid decline ≥ 0.5 log IU/mL, Slow decline < 0.5 log IU/mL]
N/A: no statistics are computed because HBsAg loss at two year is constant.

predictive for HBsAg loss. Similarly, we found that HBeAg positive patients with a rapid decline of HBsAg level more than 0.5 log IU/mL at 1 year after treatment achieved HBsAg loss (1 of 3 patients with lamivudine therapy and 2 of 6 patients with entecavir), although there was no associated HBeAg seroconversion. However, in patients with HBeAg seroconversion, the mean HBsAg decline at 96 weeks after treatment was significantly more than in those with no HBeAg seroconversion. This was similar to two previous observational studies in HBeAg-positive patients on entecavir therapy which noted that in patients with HBeAg loss/seroconversion there was a greater decline of HBsAg levels than in those without HBeAg loss[11,12]. Moreover, in HBeAg negative patients, the rapid decline of HBsAg level at one year after treatment was predictive of HBsAg loss also (all 3 patients in the lamivudine group and 1 of 3 patients in the telbivudine group). No patients with a slow decline of HBsAg level achieved an HBsAg loss. Although the mechanism of HBsAg decline during NUCs therapy is unclear, it may be hypothesized that the reduction of HBsAg level reflects a better degree of host immune control against the virus, or even a decrease in the amount of intrahepatic cccDNA. From a conceptual viewpoint, it is known that NUCs only block the reverse transcriptase enzyme, and by so doing diminish HBV DNA synthesis without a direct effect on cccDNA. Therefore, the observation that Peg-IFN produces a more pronounced HBsAg decline than NUCs do is reasonable, because Peg-IFN can induce apoptosis or necrosis in HBV infected hepatocytes. On the other hand, the early decline of HBsAg in patients receiving NUCs may be attributed to the restoration of the host immune response against HBV, as reflected by the pretreatment ALT level. This hypothesis was substantiated by the study by Reijnders et al. in HBeAg (+) or (-) patients[11], who received 48 weeks of either PEG-IFN or ETV. They found that a decline of HBsAg in HBeAg-positive CHB was primarily confined to ETV-treated patients with a baseline serum ALT >2 ULN, and could be attributed to a large extent to patients achieving HBeAg loss. That finding suggested that the presence of an active preexisting immune response against HBV is required to lower HBsAg levels for patients treated with ETV. In our study, however, we found that baseline ALT was not associated with HBeAg seroconversion and HBsAg loss. This could be due to the small number of subjects to detect significant difference. There were limitations in our study. First, the study was a retrospective design, and there was small sample size in each arm of treatment. Second, the follow-up period was under two years. It would have been better to compile the results at the end of the third or the fifth years. Third, we did not carry out genotyping before starting anti-viral medication. Thus, the implications of the different patterns of HBsAg decline as identified in our study require future prospective investigations before becoming applicable to clinical practice. In any cases, one data help support HBsAg quantitation as an additional tool for monitoring antiviral therapy.

In summary, The results from this analysis of HBsAg kinetics during nucleos(t)ide analog treatment showed that prolonged and effective viral suppression could lead to HBsAg clearance. Baseline HBsAg level < 1,000 IU/mL was associated with HBsAg loss, and rapid on-treatment declines in HBsAg levels occurring at one year were associated with future HBsAg clearance.

REFERENCES

1. Dienstag JL. Hepatitis B virus infection. N Engl J Med 2008;359(14):1486-500.
2. Chan HL, Wong VW, Tse AM, *et al.* Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol 2007;5:1462-8.
3. Brunetto MR, Moriconi F, Bonino F, *et al.* Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 2009;49:1141-50.
4. Sonneveld MJ, Rijckborst V, Boucher CA, Hansen BE, Janssen HL. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. Hepatology 2010;52:1251-7.
5. 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.
6. Gane E, Heathcote EJ, Marcellin P, *et al.* HbSAg kinetics of decay and baseline charaterristics of HBeAg positive patients with chronics hepatitis B following 3 years of tenofovir disoproxilfumarate (TDF) treatment. J Hepatol 2010;52:S388.
7. Maynard M, Parvaz P, Durantel S, *et al.* Sustained HBs seroconversion during lamivudine and adefovir dipivoxil combination therapy for lamivudine failure. J Hepatol 2005;42: 279-81.
8. Su TH, Hsu CS, Chen CL, Liu CH, Huang YW, Tseng TC, *et al.* Serum hepatitis B surface antigen concentration correlates

with HBV DNA level in patients with chronic hepatitis B. Antivir Ther 2010;15:1133-9.
9. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, *et al.* Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology 2011;140:132-43.
10. Wursthorn K, Jung M, Riva A, *et al.* Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen positive patients. Hepatology 2010;52:1611-20.
11. Reijnders JG, Rijckborst V, Sonneveld MJ, *et al.* Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir. J Hepatol 2011;54:449-54.
12. Lee JM, Ahn SH, Kim HS, Park H, Chang HY, Kim do Y, *et al.* Quantitative hepatitis B surface antigen and hepatitis B e antigen titers in prediction of treatment response to entecavir. Hepatology 2011;53:1486-93.