

## Original article

# Serum levels of surface large envelope proteins: prognostic markers for hepatitis B e antigen-negative patients with adefovir dipivoxil treatment

Xiao Fan<sup>1</sup>, Dong Fang<sup>1,2,3</sup>, Du Bin<sup>1,4</sup>, Li Xueyong<sup>1,4</sup>, Cheng Jun<sup>1</sup> and Wei Hongshan<sup>1\*</sup>

<sup>1</sup>Institute of Infectious Disease, Beijing Ditan Hospital, Capital Medical University, Beijing, China

<sup>2</sup>First Affiliated Hospital of Shanxi Medical University, Taiyuan, China

<sup>3</sup>Department of Gastroenterology, Shanxi Children's Hospital, Taiyuan, China

<sup>4</sup>Second Affiliated Hospital of Harbin Medical University, Haerbin, China

\*Corresponding author: e-mail: drliver@163.com

**Background:** The present investigation was undertaken to evaluate the prognostic role of pretreatment serum hepatitis B virus (HBV) surface large envelope protein (LHBs) levels in the curative effects after 48-week adefovir dipivoxil (ADV) treatment.

**Methods:** A total of 128 patients received ADV once daily for 48 weeks. Serum levels of LHBs were detected by ELISA. Real-time quantitative PCR was used to analyse HBV genotype and HBV DNA copies in serum. Receiver operating characteristic (ROC) curve analysis was performed to assess the optimal cutoff value of pretreatment LHBs for predicting the curative effects of ADV treatment.

**Results:** After ADV treatment for 48 weeks, viral response and partial response were 31.4% (16/51) and 29.4% (15/51), respectively, in patients from the hepatitis B e antigen (HBeAg)-positive group; viral response

and partial response were 39.7% (27/68) and 39.7% (27/68), respectively, in patients from the HBeAg-negative group. HBeAg-negative patients with high serum levels of LHBs had low response rates to antiviral therapy. ROC curve analysis showed that HBeAg-negative patients with serum LHBs levels  $\geq 3.889$   $\mu\text{g/ml}$  at baseline predicted non-response to antiviral therapy. The sensitivity was 42.5% and specificity was 92.86%. Among a total of 19 patients with high serum levels of LHBs ( $\geq 3.889$   $\mu\text{g/ml}$ ) at baseline, only 2 (11%) patients responded to antiviral therapy. There was no correlation observed between HBV genotype and effects of ADV treatment.

**Conclusions:** HBeAg-negative patients with high serum levels of LHBs ( $\geq 3.889$   $\mu\text{g/ml}$ ) at baseline should not be recommended to receive ADV treatment.

## Introduction

Approximately 350 million people are chronically infected with hepatitis B virus (HBV) worldwide, among whom approximately 200 million are in China. HBV infection can persist for the whole lifetime, often leading to severe consequences, such as liver failure, cirrhosis and hepatocellular carcinoma [1]. Chronic infection with HBV is one of the most important public health problems.

The treatment of chronic hepatitis B (CHB) is still a challenge, despite advances in therapeutic medicines, because some patients are unable to achieve sustained virological response [2]. The long-term goals of therapy for CHB are to reduce serum HBV DNA levels to below detectable limits, to slow the progression of liver fibrosis and, ultimately, to decrease the incidence of

hepatocellular carcinoma [3]. Therapy with interferon or nucleoside analogues alone or in combination can be effective against HBV; however, side effects of interferon and the emergence of nucleoside-resistant mutants often limit treatment outcomes [4].

Adefovir dipivoxil (ADV) has been used alone or together with lamivudine to suppress lamivudine-resistant HBV. After 1 year of therapy, a median 3.5–4.0  $\log_{10}$  reduction in serum HBV DNA was observed [5]. ADV was advantageous and safe for oral administration, but induced a sustained response after withdrawal of therapy only in a minority of patients. In general, the antiviral responses to ADV in clinical practice varied across individual patients, and some patients had no long-term effects at all [6]. Heretofore, no appropriate prognostic

marker was available to predict the long-term effects of antiviral therapy, except for repeating expensive detection techniques of HBV DNA, which imposes a heavy burden on patients, especially those in developing countries.

Detectable HBV DNA in plasma or serum is one of the criteria for CHB in all guidelines. There is general agreement among practice guidelines that the decision to initiate therapy should be based on the demonstration of active viral replication (defined as an HBV DNA level of  $\geq 100,000$  copies/ml in hepatitis B e antigen (HBeAg)-positive CHB patients and moderate to severe disease, as evidenced by persistent alanine aminotransferase (ALT) increase (3–6 months) and/or histological demonstration of moderate to severe hepatitis. Measurement of HBV DNA is more crucial for the diagnosis and management of HBeAg-negative CHB patients, as it is the only marker of viral replication that can be monitored [7].

Although viral load measurement plays an important role during therapy, significant variability in quantification among different assays occurs randomly, despite the standardization of reporting units and the finding of generally good correlation between different assays [8,9]. The HBV genotype is another variable that might influence quantification by different methods [10]. The specificity, reproducibility and false-positive rate of results could influence HBV DNA detection [11]. Above all, high cost, requirements of circumstance, complicated devices and advanced techniques limit its use in developing country.

In recent years, we have performed analyses on hundreds of serum samples from CHB patients. Our results demonstrated that serum levels of surface large envelope proteins (LHBs) in HBeAg-negative patients correlated with HBV DNA copies [12]. Consequently, the aim of the present study is to assess the relationship between serum LHBs levels and HBV DNA copies, in order to evaluate the prognostic role of serum LHBs to long-term effects of ADV antiviral therapy.

## Methods

### Study design

A total of 128 consecutive patients with CHB were recruited, and provided written informed consent. All patients received 10 mg ADV (Shanghai Modern Pharmaceutical Co., Ltd, Shanghai, China) once daily after primary laboratory screening was completed. The study comprised 48 weeks of treatment. After antiviral therapy for 12, 24, 36 and 48 weeks, serum HBV DNA and LHBs were detected repeatedly. Approval was obtained by the Medical Ethics Committee of Beijing Ditan Hospital (Beijing, China).

### Patients

Patients were divided into two groups, the HBeAg-positive and HBeAg-negative group. All patients were

positive for hepatitis B surface antigen (HBsAg) for  $\geq 6$  months, their serum HBV DNA level was  $>500,000$  copies/ml and serum ALT level was 3–10 $\times$  the upper limit of the normal range. Based on a liver biopsy within the previous 12 months, all patients were consistent with the presence of CHB.

Exclusion criteria was decompensated liver disease, coexisting serious medical or psychiatric illness, neutrophil count of  $<1,500$  cells/mm<sup>3</sup>, platelet count of  $<90,000$  cells/mm<sup>3</sup>, serum creatinine level that was  $>1.5\times$  the upper limit of the normal range, history of alcohol or drug abuse within 1 year before entry and coinfection with hepatitis C virus, hepatitis delta virus or HIV. Previous treatment for CHB was permitted, but not within 6 months before study participation.

### Efficacy measures

Efficacy analyses included all patients who had received at least one dose of medication. Serum HBeAg and HBV DNA were measured with the AxSYM test (Abbott Laboratories, Abbott Park, IL, USA) and the Cobas® Amplicor HBV Monitor Test (Roche Diagnostics, Basel, Switzerland), respectively. Except for pretreatment assessment, the efficacy measures were evaluated after 12, 24, 36 and 48 weeks treatment, including the combined response, such as HBeAg seroconversion, the normalization of ALT levels (data not shown), the reduction of HBV DNA levels and HBsAg seroconversion.

### Serum LHBs measurement

The serum levels of LHBs were measured with ELISA kit (Beijing Hotgen Biotech Co., Ltd, Beijing, China), which was performed using THERMO Varioskan Flash (Thermo Scientific, Waltham, MA, USA). The critical value of LHBs absorbance was defined as 0.105, which was equal to 1 ng/ $\mu$ l. It was judged as a negative result when the value of samples absorbance was  $<0.105$ .

### Genotype analyses

Genotype analysis of HBV DNA was performed at baseline with serum samples from all patients, which was done by fluorescent hybridization probes (ZJ Bio-Tech, Shanghai, China) and a real-time PCR machine (ABI 7500; Applied Biosystems, Foster City, CA, USA).

### Statistical analyses

Patients per treatment group provided the study with a two-sided test in order to detect a difference in HBV DNA response rates. An overall significance level of 0.05 was chosen because of the primary end points. For each treatment group, response rates were computed with corresponding 95% confidence intervals. The Student's *t*-test was used to identify statistical difference between the two groups. The receiver operating characteristic

(ROC) curve analysis was applied to make a certain optimal value of LHBs to predict the antiviral effects. SPSS 11.0 (SPSS Inc., Chicago, IL, USA) was used for descriptive statistics and analyses.

## Results

### Characteristics of the patients

All 128 patients received ADV treatment at baseline, but only 120 patients were followed-up for 48 weeks. After 24 weeks of ADV treatment, six patients had virological response, but with rebound of HBV replication at 48 weeks. These six patients were excluded from our analysis.

Of the 120 patients included in the analyses and who were assigned to receive ADV treatment, 52 patients were HBeAg-positive and 68 patients were HBeAg-negative. Baseline demographics and other characteristics are listed in Table 1.

### Virological response

A viral partial response was defined as reduction in serum HBV DNA by  $\geq 2 \log_{10}$ , but with detectable ( $\geq 1,000$  copies/ml) HBV DNA. A viral response was defined as undetectable ( $< 1,000$  copies/ml) serum HBV DNA. A viral non-response was defined as a serum HBV DNA level reduction  $< 2 \log_{10}$  copies/ml after 48 weeks of antiviral therapy. In the HBeAg-positive group, 29.4% (15/51) of patients had a viral partial response, 31.4% (16/51) of those had a viral response, and 39.2% (20/51) of patients had a non-response. Among HBeAg-negative patients, 39.7% (27/68) had a viral partial response, 39.7% (27/68) of those had a viral response and 20.6% (14/68) of patients had a viral non-response (Table 2 and Figure 1).

### ROC curve analysis of serum LHBs levels

ROC curve analysis was performed to assess the optimal cutoff value of LHBs to predict the effect of ADV treatment. The analysis indicated that HBeAg-negative patients with serum LHBs levels  $\geq 3.889 \mu\text{g/ml}$  at baseline might have no response to ADV treatment. Of a total of 19 patients with serum LHBs  $\geq 3.889 \mu\text{g/ml}$ , 17 (89%) patients had no viral response to ADV treatment. The prognostic sensitivity was 42.5% and specificity was 92.86%. After 12 weeks of ADV treatment, among a total of 18 patients with serum LHBs levels  $\geq 2.855 \text{ ng/ml}$ , only 2 patients had a viral response to ADV treatment. The prognostic sensitivity of viral non-response was 40% and specificity was 92.86% (Figure 2 and Table 3). HBeAg-positive patients with serum LHBs levels  $\geq 3.808 \mu\text{g/ml}$  after 24 weeks of ADV treatment might have a poor viral response. The prognostic sensitivity was 20% and specificity was 93.75% (Figure 2 and Table 4).

**Table 1.** Baseline characteristics of the patients

Characteristic	HBeAg-positive (n=51)	HBeAg-negative (n=68)
Mean age, years (range)	34 (25–55)	33 (26–54)
Male sex, n (%)	37 (71)	54 (79)
Mean weight, kg (range)	65 (39–130)	59 (38–129)
HBV genotype		
B, n (%)	19 (37.3)	29 (42.6)
C, n (%)	31 (60.8)	36 (52.9)
Other/unknown, n (%)	1 (1.9)	3 (4.5)
Mean serum HBV DNA, $\log_{10}$ copies/ml ( $\pm$ SD)	7.05 (1.03)	6.84 (1.25)
Median serum HBV DNA, $\log_{10}$ copies/ml (range)	7.09 (3.21–8.61)	6.37 (3.45–9.35)

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

**Table 2.** Efficacy results at week 48

Variable	HBeAg-positive (n=51)	HBeAg-negative (n=68)
Viral partial response, n (%)	15 (29.4)	27 (39.7)
Viral response, n (%)	16 (31.4)	27 (39.7)
Viral non-response, n (%)	20 (39.2)	14 (20.6)

A viral partial response was defined as a reduction in serum hepatitis B virus (HBV) DNA by  $\geq 2 \log_{10}$  copies/ml, but with HBV DNA  $\geq 1,000$  copies/ml. A viral response was defined as serum HBV DNA  $< 1,000$  copies/ml. A viral non-response was defined as serum HBV DNA level remaining  $> 5 \log_{10}$  copies/ml through 48 weeks. HBeAg, hepatitis B e antigen.

### Genotype analyses

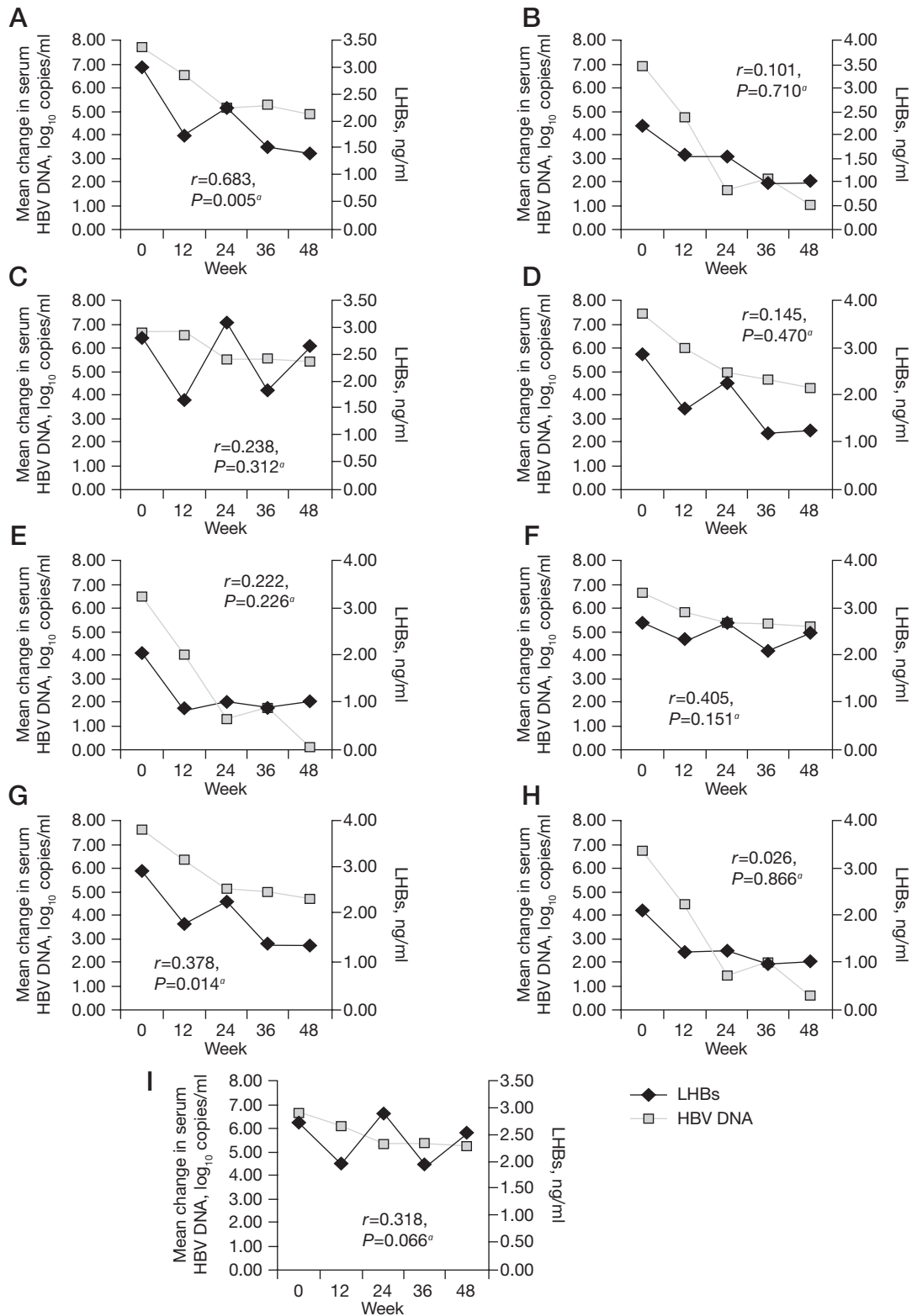
In our study, there were no significant differences in viral response between patients with genotypes A and B after 48 weeks of ADV treatment (data not shown).

## Discussion

The present investigation demonstrated, firstly, that treatment with ADV resulted in improvements of virological, serological and biochemical markers for patients with CHB, but no differences were observed between genotype B and C. Secondly, the serum levels of LHBs in ADV pretreatment patients correlated with serum HBV DNA copies, especially to those of HBeAg-negative patients. Thirdly, the pretreatment serum levels of LHBs might serve as a prognostic marker in evaluating the effects of ADV antiviral treatment.

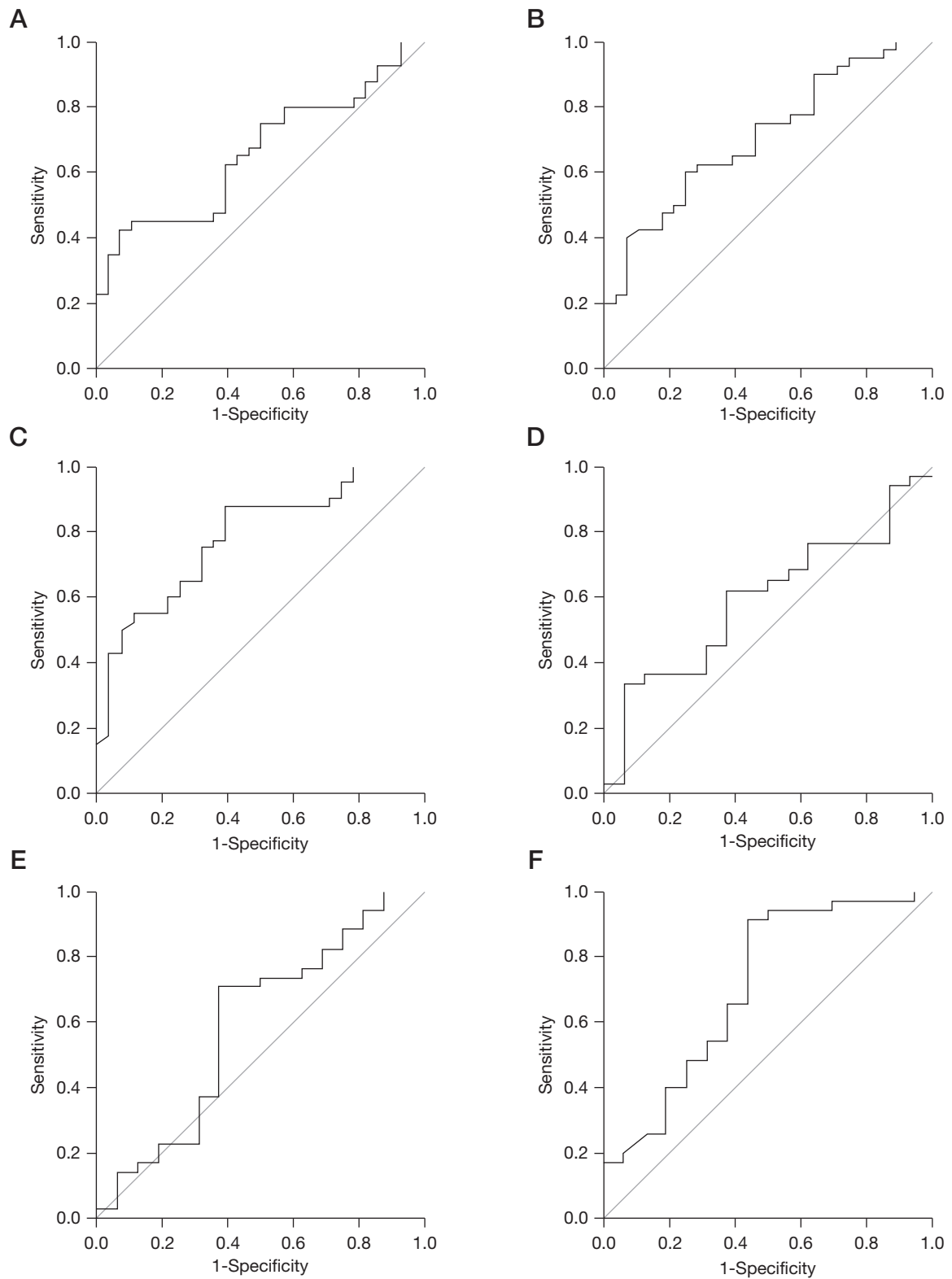
Among HBeAg/antibodies against HBeAg seroconversion, serum HBV DNA and ALT, the best viral marker for the management of HBV disease is serum HBV DNA. At present, measurement of serum HBV DNA level has been used as the primary method in virological monitoring of hepatitis B antiviral therapy in clinical practice, but quantification of HBV DNA levels

Figure 1. Serum HBV DNA and LHBs levels from baseline to week 48



(A) Viral partial response patients who were hepatitis B e antigen (HBeAg)-positive (n=15). (B) Viral response patients who were HBeAg-positive (n=16). (C) Viral non-response patients who were HBeAg-positive (n=20). (D) Viral partial response patients who were HBeAg-negative (n=27). (E) Viral response patients who were HBeAg-negative (n=27). (F) Viral non-response patients who were HBeAg-negative (n=14). (G) Viral partial response hepatitis B virus (HBV) patients (n=42). (H) Viral response HBV patients (n=43). (I) Viral non-response HBV patients (n=34). <sup>a</sup>The correlation between serum levels of serum large envelope proteins (LHBs) at baseline and HBV DNA copies at week 48.

Figure 2. Receiver operating characteristic curve analyses



(A) Hepatitis B e antigen (HBeAg)-negative patients at baseline. (B) HBeAg-negative patients at week 12. (C) HBeAg-negative patients at week 24. (D) HBeAg-positive patients at baseline. (E) HBeAg-positive patients at week 12. (F) HBeAg-positive patients at week 24.

**Table 3.** ROC analysis of LHBs level in HBeAg-negative patients

Variable	Baseline	Week 12	Week 24
Area	0.658	0.707	0.782
SE	0.066	0.063	0.056
95% CI	0.5291–0.7800	0.5841–0.8293	0.6728–0.8915
<i>P</i> -value	0.027	0.004	<0.0001
Sensitivity, %	42.50	40.00	50
Specificity, %	92.86	92.86	92.86
Cutoff	3.889	2.855	2.455
Viral response, <i>n</i> % <sup>a</sup>	2 (11)	2 (11)	2 (9)
Viral non-response, <i>n</i> % <sup>a</sup>	17 (89)	16 (89)	20 (91)

<sup>a</sup>Patients with viral response or non-response greater than or equal to the cutoff value. CI, confidence interval; HBeAg, hepatitis B e antigen; LHBs, serum large envelope proteins; ROC, receiver operating characteristic.

**Table 4.** ROC analysis of serum LHBs level in HBeAg-positive patients

Variable	Baseline	Week 12	Week 24
Area	0.596	0.595	0.711
SE	0.083	0.092	0.084
95% CI	0.434–0.759	0.414–0.776	0.5454–0.8760
<i>P</i> -value	0.273	0.282	0.017
Sensitivity, %	37.1	37.1	20
Specificity, %	87.5	0.688	93.75
Cutoff	4.124	1.0218	3.808
Viral response, <i>n</i> % <sup>a</sup>	2 (13)	5 (28)	1 (13)
Viral non-response, <i>n</i> % <sup>a</sup>	13 (87)	13 (72)	7 (87)

<sup>a</sup>Patients with viral response or non-response greater than or equal to the cutoff value. CI, confidence interval; HBeAg, hepatitis B e antigen; LHBs, surface large envelope proteins; ROC, receiver operating characteristic.

is expensive and not readily available or affordable in many countries where HBV is prevalent [13]. Although recently HBV serum level of HBsAg was used to evaluate the effect of antiviral therapy, the clearance of serum HBsAg was much slower than that of serum HBV DNA [14]. The low cost and readily operated method, which is correlated with HBV DNA, is still absent.

In order to make certain the optimal cutoff value of serum levels of LHBs for prognosticating the curative effect of antiviral therapy, we performed a ROC analysis. According to the present results, serum LHBs levels of patients who were HBeAg-negative at baseline or after ADV treatment for 12 and 24 weeks were correlated with the effect of antiviral therapy at week 48. In the HBeAg-positive group, patients with higher serum levels of LHBs ( $\geq 3.808$   $\mu\text{g/ml}$ ) after 24 weeks of antiviral therapy had a poorer response to ADV treatment. Among a total of eight cases of HBeAg-positive patients with serum levels of LHBs  $\geq 3.808$   $\mu\text{g/ml}$  after a 24-week ADV treatment, only one (13%) patient had a response to antiviral therapy after 48 weeks of ADV-treatment. But among 43 cases, HBeAg-positive patients with serum levels of LHBs  $< 3.808$   $\mu\text{g/ml}$  after 24 weeks of ADV treatment, 35% (15/43) patients responded to

antiviral therapy after a 48-week ADV treatment. The response rate to antiviral therapy was a significantly different between the two groups ( $P < 0.05$ ).

The most important finding of the present investigation was the correlation between serum levels of LHBs and the effects of ADV treatment, which was stronger in HBeAg-negative patients. The ROC analysis revealed that the serum levels of LHBs ( $\geq 3.889$   $\mu\text{g/ml}$ ) at baseline was the best predictors of antiviral response in HBeAg-negative patients (area under ROC curve = 0.658), but this trend was not observed in HBeAg-positive patients. In those patients with serum levels of LHBs  $\geq 3.889$   $\mu\text{g/ml}$  at baseline, most had a poor response to antiviral therapy. A total of 19 patients had serum levels of LHBs  $\geq 3.889$   $\mu\text{g/ml}$  at baseline, only 11% (2/19) of whom responded to antiviral therapy at week 48. The present results suggested that HBeAg-negative CHB patients with serum LHBs  $\geq 3.889$   $\mu\text{g/ml}$  should not be recommended to receive ADV treatment.

Based on our results, compared with those of HBeAg-positive patients, there is a better correlation between the serum level of LHBs and virological response in HBeAg-negative patients. This difference might partly be the result of different replication characteristics. CHB

is characterized by an early replicative phase (HBeAg-positive) and a late low or non-replication phase with HBeAg seroconversion and liver disease remission (HBeAg-negative) [15,16]. The follow-up time and the quantity of enrolled patients might also influence the results.

HBV is an unusual virus because its surface proteins are not only incorporated into virion envelopes, but also generate empty subviral spherical or filamentous particles without nucleocapsids in an intracellular compartment, which form in great excess over virions. The three envelope proteins are encoded by a single open reading frame, using three in-frame start codons [17]. Among the three envelope proteins, it is not known why only the LHBs might be used as a biomarker to prognosticate the effects of antiviral therapy.

In the serum of CHB patients, there are three types of virus particles, including 22 nm spherical subviral particles, filaments with various lengths and 42 nm Dane's particles; however, only the 42 nm Dane's particles contain the HBV genome [18,19]. Even more important, the three types of particles contain three different envelope proteins (large, middle and small) with different proportions of each. The 22 nm spherical subviral particles are the most abundant type (10,000-fold more than Dane's particles), assembled mainly with small envelope proteins and a small quantity of middle envelope proteins. LHBs also participate in assembling the 22 nm filaments particles, but are more abundant in Dane's particles [20]. Therefore, it is common that HBV DNA is decreased <1,000 copies/ml after long-term antiviral therapy, but HBsAg (small envelope protein) in serum still maintains high levels [21].

According to other reports, all three envelope proteins contain a common N-glycosylation site in the S domain, but with different glycosylation patterns. In the presence of *N*-butyl-deoxynojirimycin (an inhibitor of  $\alpha$ -glucosidase), three envelope proteins are retained in different compartments in hepatocytes. Middle protein is localized at lysosomal vesicles, but the small and large proteins are secreted [22]. N-linked oligosaccharides play many roles in the fate and functions of glycoproteins [23]. In the process of morphogenesis, although small proteins are essential and sufficient for budding, the assembly of Dane's particles depends on LHBs, which are correctly folded in endoplasmic reticulum [24,25].

From our results and other reports, we speculated that only with a proper proportion of LHBs, MHBs and SHBs can the Dane's particles be assembled with three envelope proteins. In addition, with less LHBs, the assembled particles mainly were filaments with different lengths. Finally, without LHBs, with very few, or with a large amount of surface small envelope proteins and a small quantity of surface medium envelope

proteins, the assembled particles mainly were 22 nm. Consequently, among three envelope proteins, only LHBs was related with Dane's particles, the true virus particles, which contained HBV DNA. It is easy to comprehend that the serum level of LHBs is the best correlated marker with HBV DNA copies.

In contrast to other results [26,27], our data did not show the relationship between HBV genotype and the effects of ADV treatment. The different results might be because the number of patients who received ADV treatment and the time of treatment are different. Furthermore, most clinical investigations did not show the difference of effect between genotype B and C in ADV-treated patients, although these differences were observed between genotype A and D [28].

On the basis of the present data, pretreatment serum levels of LHBs could be used to determine which patient was suitable for ADV antiviral therapy. The patients with high serum levels of LHBs ( $\geq 3.889 \mu\text{g/ml}$ ) at baseline, especial those of HBeAg-negative patients, should not be recommended to receive ADV treatment. Because all oral nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) have the same molecular mechanism targeting HBV, the relationship between sera levels of LHBs and antiviral effects of other NRTIs needs to be further elucidated.

## Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (numbers 30671875 and 30872243)

## Disclosure statement

The authors declare no competing interests.

## References

1. Ying C, Li Y, Leung C-H, Robek MD, Cheng Y-C. Unique antiviral mechanism discovered in anti-hepatitis B virus research with a natural product analogue. *Proc Natl Acad Sci U S A* 2007; **104**:8526–8531.
2. Tong MJ, Hsien C, Hsu L, Sun HE, Blatt LM. Treatment recommendations for chronic hepatitis B: an evaluation of current guidelines based on a natural history study in the United States. *Hepatology* 2008; **48**:1070–1078.
3. 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.
4. Lok AS, Zoulim F, Locarnini S, *et al.* Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**:254–265.
5. Chan HL, Heathcote EJ, Marcellin P, *et al.* Treatment of hepatitis B e antigen positive chronic hepatitis with telbivudine or adefovir: a randomized trial. *Ann Intern Med* 2007; **147**:745–754.
6. Palumbo E. New drugs for chronic hepatitis B: a review. *Am J Ther* 2008; **15**:167–172.

7. Valsamakis A. Molecular testing in the diagnosis and management of chronic hepatitis B. *Clin Microbiol Rev* 2007; **20**:426–439.
8. Hui CK, Bowden S, Zhang HY, *et al.* Comparison of real-time PCR assays for monitoring serum hepatitis B virus DNA levels during antiviral therapy. *J Clin Microbiol* 2006; **44**:2983–2987.
9. Plentz A, Koller G, Weinberger KM, Jilg W. Precision and stability of hepatitis B virus DNA levels in chronic surface antigen carriers. *J Med Virol* 2004; **73**:522–528.
10. Laperche S, Thibault V, Bouchardeau F, *et al.* Expertise of laboratories in viral load quantification, genotyping, and precore mutant determination for hepatitis B virus in a multicenter study. *J Clin Microbiol* 2006; **44**:3600–3607.
11. Yao JD, Beld MG, Oon LL, *et al.* Multicenter evaluation of the VERSANT hepatitis B virus DNA 3.0 assay. *J Clin Microbiol* 2004; **42**:800–806.
12. Wei HS, Huang YB, Song SJ, Dong QM, Li GL, Cheng J. The relationship between serum LHBs and HBV DNA copies in patients with HBeAg-negative chronic hepatitis B. *Zhonghua Gan Zang Bing Za Zhi*. 2006; **14**:543–544. Chinese.
13. Pawlotsky JM, Dusheiko G, Hatzakis A, *et al.* Virologic monitoring of hepatitis B virus therapy in clinical trials and practice: recommendations for a standardized approach. *Gastroenterology* 2008; **134**:405–415.
14. Borgniet O, Parvaz P, Bouix C, *et al.* Clearance of serum HBsAg and anti-HBs seroconversion following antiviral therapy for chronic hepatitis B. *J Med Virol* 2009; **81**:1336–1342.
15. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**:335–352.
16. Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. *Semin Liver Dis* 2006; **26**:130–141.
17. Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-s sequence. *J Virol* 1984; **52**:396–402.
18. Gerin JL, Ford EC, Purcell RH. Biochemical characterization of Australia antigen. Evidence for defective particles of hepatitis B virus. *Am J Pathol* 1975; **81**:651–668.
19. Hruska JF, Robinson WS. The proteins of hepatitis B Dane particle cores. *J Med Virol* 1977; **1**:119–131.
20. Bruss V. Envelopment of the hepatitis B virus nucleocapsid. *Virus Res* 2004; **106**:199–209.
21. Liaw YF. Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. *Liver Int* 2009; **29 Suppl 1**:100–107.
22. Mehta A, Lu X, Block TM, Blumberg BS, Dwek RA. Hepatitis B virus (HBV) envelope glycoproteins vary drastically in their sensitivity to glycan processing: evidence that alteration of a single N-linked glycosylation site can regulate HBV secretion. *Proc Natl Acad Sci U S A* 1997; **94**:1822–1827.
23. Frank CG, Sanyal S, Rush JS, Waechter CJ, Menon AK. Does Rft1 flip an N-glycan lipid precursor? *Nature* 2008; **454**:E3–E4.
24. Mehta A, Zitzmann N, Rudd PM, Block TM, Dwek RA. Alpha-glucosidase inhibitors as potential broad based antiviral agents. *FEBS Lett* 1998; **430**:17–22.
25. Patient R, Hourieux C, Sizaret PY, Trassard S, Sureau C, Roingeard P. Hepatitis B virus subviral envelope particle morphogenesis and intracellular trafficking. *J Virol* 2007; **81**:3842–3851.
26. Rodriguez-Frias F, Jardi R, Schaper M, *et al.* Adefovir for chronic hepatitis B treatment: identification of virological markers linked to therapy response. *Antivir Ther* 2008; **13**:991–999.
27. Buti M, Elefsiniotis I, Jardi R, *et al.* Viral genotype and baseline load predict the response to adefovir treatment in lamivudine-resistant chronic hepatitis B patients. *J Hepatol* 2007; **47**:366–372.
28. Palumbo E. Hepatitis B genotypes and response to antiviral therapy: a review. *Am J Ther* 2007; **14**:306–309.