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ORIGINAL ARTICLE

Baseline HBsAg levels associated with HBsAg loss in HBeAg-negative chronic hepatitis B infection with persistently normal alanine aminotransferase

Li Chen[a,*,1], Junjie Shi[a,1], Zhonghua Lu[b,1], Yun Ye[a], Xinbei Zhou[a], Youwen Tan[a,*]

[a] *Department of Hepatology, The Third Hospital of Zhenjiang Affiliated Jiangsu University, Zhenjiang, 212003, PR China*
[b] *Department of Liver Disease, Wuxi No. 5 People's Hospital Affiliated to Jiangnan University, Wuxi, 214000, PR China*

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KEYWORDS
Chronic hepatitis B;
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HBsAg loss;
Chinese

Summary
Background: We aimed to assess the long-term outcomes of e antigen-negative chronic hepatitis B (CHB) infection with low hepatitis B virus (HBV) DNA level (< 200 IU/mL) and persistently normal alanine aminotransferase (PNALT) and to explore the factors associated with the results.
Methods: This retrospective cohort study enrolled consecutive baseline CHB patients with PNALT from January 2005 to June 2008. In total, 252 e antigen-negative CHB patients with PNALT and low HBV DNA level (< 200 IU/mL) were enrolled, of whom 188 were eligible for this analysis. Among the 188 patients, 131 were followed up more than twice per year and 57 were followed up at least once per year, with a median follow-up period of 102 (73—123) months.
Results: Of 188 patients, 16 had HBV DNA level of > 200 IU/mL and PNALT, 164 had HBV DNA level of < 200 IU/mL and PNALT and 8 had HBV DNA level of > 200 IU/mL and elevated ALT level, of which 3 used an antiviral drug during follow-up. Twelve of 164 experienced HBsAg loss. Cox regression analysis suggested that baseline HBsAg levels were associated with HBsAg loss in patients after follow-up, especially the baseline HBsAg levels of < 200 IU / mL, which is a risk factor for HBsAg loss. The AUC of baseline HbsAg level in the e antigen-negative CHB group was 0.772 (cutoff value 426, $P < 0.001$). The cumulative probability of HBsAg loss in the HBsAg < 400 IU/L group was 20% (7/35), which ws higher than that in the HBsAg ≥ 400 IU/L group (3.88%; 5/129; X2 = 11.75, $P = 0.0006$).
Conclusion: The e antigen-negative CHB infection with low HBV DNA level (< 200 IU/mL) and PNALT will progress to chronic hepatitis, although the probability of its occurrence is low.

* Corresponding author.
E-mail addresses: zjsychenli826@sina.com (L. Chen), tyw915@sina.com (Y. Tan).
[1] These authors contributed equally to this work.

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Spontaneous HBsAg loss may not occur frequently because the manifested cumulative probability of HBsAg loss was higher in the HBsAg < 400 IU/L group than in the HBsAg ≥ 400 IU/L group.

Introduction

Hepatitis B virus (HBV) infection is a serious health problem in China, and it causes cirrhosis and complications, from hepatocellular carcinoma (HCC) to end-stage liver disease [1], depending on the host immune response and HBV factors. The proportion of cirrhosis and HCC cases caused by HBV infection were 60% and 80% respectively. The 2006 Serum Hepatitis B Epidemiology Survey in China showed that 7.18% of the general population in the age group of 1—59 years were carriers of HBsAg [2].

The nature of HBV infection is divided into five phases:

- HBeAg-positive chronic infection;
- HBeAg-positive chronic hepatitis;
- HBeAg-negative chronic infection;
- HBeAg-negative chronic hepatitis, and;
- HBsAg-negative phase.

The new nature of HBV infection is based on the description of the two main characteristics of chronicity, i.e., infection or hepatitis, based on the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) levels, and eventually the presence or absence of liver inflammation.

The previously termed ''inactive carrier'' phase was renamed e antigen-negative chronic hepatitis B (CHB) infection, which is characterized by low HBV viral replication or persistently normal ALT (PNALT). These patients are generally considered to have a good prognosis, with the risk of developing cirrhosis and HCC being very low. Low HBV viral replication may delimit HBV DNA levels to < 2000 IU/mL and occasionally to ≤ 200 IU/mL. More evidence suggests that low HBV viral levels are associated with a benign outcome, accompanied by PNALT, minimal hepatic necroinflammatory activity, and low fibrosis, as in an e antigen-negative CHB infection. These patients have a low risk of progression to cirrhosis or HCC if they remain in this phase.

However, if we define low HBV viral replication as < 200 IU/mL, what is the long-term outcome of e antigen-negative CHB with PNALT?

Materials and methods

Ethics statement

This study was approved by the Medical Ethics Committee of The Third Hospital of Zhenjiang Affiliated Jiangsu University, and a written informed consent was obtained from each patient prior to participation. The study was conducted in accordance with the Declaration of Helsinki.

Patients

This was a retrospective cohort study. The flow chart of the study design is shown in Fig. 1. From January 2005 to June 2008, consecutive baseline CHB patients with PNALT were enrolled at the Liver Clinic and Department of Hepatology, The Third Hospital of Zhenjiang Affiliated Jiangsu University. The patients were examined every 3—6 months or more often if clinically indicated. At each visit, the results of liver biochemistry, ultrasonography, HBV serology, including HBsAg, HBeAg, anti-HBe and HBV DNA levels. The inclusion criteria were as follows [3]:

- HBsAg-positive for > 6 months;
- HBV DNA level of < 200 IU/mL; and;
- PNALT assessed as at least three ALT level of < 40 IU/L examined in the year [4—9].

The exclusion criteria were as follows:

- hepatitis A, C, or D or human immunodeficiency virus (HIV) coinfection;
- evidence of liver disease of another etiology;
- use of hepatotoxic drugs or regular consumption of alcohol; and;
- previous anti-viral (HBV) therapy or any liver functional protection therapy to alleviate hepatic inflammation.

In total, 225 PNALT patients were enrolled. After 3 coinfections with HAV/HEV, 25 patients were lost to follow-up and 9 patients used traditional Chinese medicine. Consequently, 188 patients were eligible for this study. Among 188 patients, 131 were followed up more than twice per year and 57 were followed up at least once per year.

After a median 102 months of follow-up (73—123 months), of the 188 patients, 16 had HBV DNA level of > 200 IU/mL and PNALT, 164 had HBV DNA level of < 200 IU/mL and PNALT, 8 had HBV DNA level of > 200 IU/mL and elevated ALT. of whom 3 were administered antiviral drug during follow-up because of biochemistry and virology test results, which was in line with the standard of antiviral therapy recommended by the 2012 EASL guidelines [10]. Of 164 patients with HBV DNA level of < 200 IU/mL and PNALT, 12 experienced HBsAg loss.

Biochemical and Serological Tests

Biochemical tests and complete blood cell count tests were performed using routine automated analyzers. The normal upper limit of the ALT level was 40 IU/L. HBsAg, HBeAg, and anti-HBe levels were assayed with commercially available enzyme-linked immunosorbent assay kits. The HBV DNA

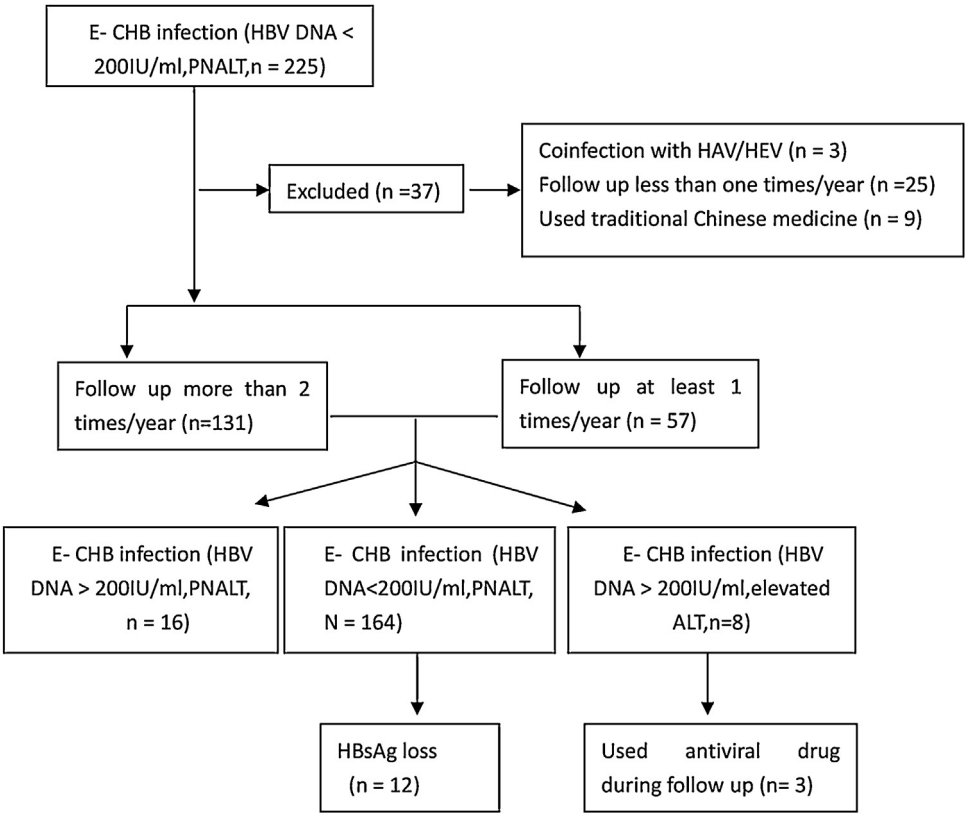

Figure 1 Flow chart of study design.

level was measured by real-time PCR, with a lower detection limit of 200 IU/mL or by COBAS TaqMan assay (sensitivity 12-IU/mL,dynamic range 6—1.10 $\times$ 10^8-IU/mL) (DaAn Gene Co, shanghai,China). HBsAg was quantified using the Architect HBsAg assay (DaAn Gene Co, Shanghai, China; dynamic range, 0.05—250.0 IU/mL).

Liver stiffness measurement

Transient elastography (FibroScan502®, Echosens, Paris, France) was performed for liver stiffness measurement (LSM) with a 3.5-MHz standard probe by a skillful operator (experience of > 10,000 measurements), who was blinded to other parameters of the patients. As previously described, the examination was performed with the patient lying down in the supine position with the right arm placed behind the head. The tip of the probe transducer was placed on the skin with adequate pressure between the ribs at the level of the right lobe of the liver. The results were expressed in kPa, and each LSM corresponded to a median of 10 validated measurements [11]. An examination was considered successful and reliable if the interquartile range (IQR)/median for LSM was $\leq$ 30% or LSM was < 7.1 kPa when the IQR/median for LSM was > 30% [12].

Statistical analysis

Results are given as median (range) or mean $\pm$ SD as percentage of patients. All data of demographic and clinical features were analyzed by the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's exact tests were performed for categorical variables, while Student's *t*-test or one-way analysis of variance was used for group comparisons of parametric quantitative data. Multivariate analysis was performed using the Cox proportional hazards regression model to evaluate factors predicting HBsAg loss. The receiver operating characteristic (ROC) curves were used to analyze predicted probabilities of the parameters. The Kaplan—Meier method was used to calculate the cumulative rate of HBsAg loss. The log-rank test was used to compare the cumulative rate of HBsAg loss groups. ROC and the Kaplan—Meier analyses were performed using MedCalc (version 10.4.7.0; MedCalc, Mariakerke, Belgium). All *P*-values were two sided.

Results

Characteristics of e antigen-negative CHB patients with PNALT after follow-up

The characteristics of 225 cases of e antigen-negative CHB with PNALT and 188 cases with CHB after follow-up are shown in Table 1. Sex (male or female); body mass index (BMI); vertical transmission of infection history (yes/no); levels of HBV DNA (undetectable; < 200; $\geq$ 200), ALT, PLT, and HBsAg (undetectable; < 200; $\geq$ 200, < 2000; $\geq$ 2000); LSM were analyzed. The differences in age and HBV DNA,

Table 1 Characteristics of E-negative CHB patients with PNALT after follow-up.

Factors	Baseline ($n = 225$)	Follow-up ($n = 188$)	P
Gender			
Man	143 (63.6%)	126 (67%)	0.467[b]
Femal	82 (36.4%)	62 (33%)	
BMI	21.22 ± 3.23	22.31 ± 3.24	0.224[a]
Vertical transmission of infection history			
Yes	117 (52%)	91 (48.4%)	0.462[b]
No	108 (48%)	97 (51.6%)	
HBV DNA (IU / mL)	235.5 ± 94.6	1253 ± 1559	**< 0.001**[a]
≧ 200	0 (0 %)	24 (12.8%)	**< 0.001**[b]
< 200	154 (68.4%)	98 (52.1%)	
undetectable	71(31.6%)	66 (35.1%)	
ALT	24.64 ± 8.32	33.25 ± 26.25	**0.002**[a]
PLT ($\times 10^9$/L)	224.35 ± 78.39	216.37 ± 90.47	0.885[b]
LSM (kpa)	4.54 ± 1.37	4.88 ± 2.37	0.104
HBsAg (IU / ml)	3425.16 ± 1544.65	2290.43 ± 1236.35	**< 0.001**[a]
≧ 2000	46 (20.4%)	25 (13.3%)	**< 0.001**[b]
< 2000, ≧ 200	143 (63.6%)	126 (67%)	
< 200	36 (16%)	25 (13.3%)	
Undectable	0 (0%)	12 (6.4%)	

Parameters are expressed as mean ± SD or number (%) PNALT: persistent normal ALT; ALT: alanine aminotransaferase; PLT: platelet; BMI: body mass index; LSM: liver stiffness measurement. The normal range of ALT is 5—40 U/L; PLT is 100—300 $\times 10^9$/L. Significance of P values in bold: significantly different between follow-up and baseline patients.

[a] One-way analysis.

[b] Pearson Chi^2.

ALT, LSM, and HBsAg levels were statistically significant ($P < 0.05$) between the baseline and follow-up groups. We observed that 24 patients had HBV DNA level of > 200 IU / mL during the follow-up period, 12 of whom (164 patients with HBV DNA level of < 200 IU / mL) experienced HBsAg loss.

Characteristics of e antigen-negative CHB patients with PNALT with HBsAg loss (or no)

A total of 164 patients had HBV DNA level of < 200 IU / mL and PNALT after follow-up, of whom 12 experienced HBsAg loss. The annual HBsAg loss was 0.83% (12/225/year). Table 2 shows the data of 12 patients who experienced HBsAg loss. BMI; vertical transmission of infection history (yes/no); levels of baseline HBV DNA (undetectable; < 200; ≥ 200), ALT, and PLT; LSM; follow-up time and baseline HBsAg level (IU / mL) (undetectable; < 200; ≥ 200, < 2000; ≥ 2000) were analyzed. The differences in age, HBV DNA levels, and baseline HbsAg levels were statistically significant ($P < 0.05$) between the no HBsAg and HBsAg loss groups. There were no differences in PLT and ALT levels and LSM. When no and HBsAg loss was considered as a binary dependent variable, we used multiple Cox regression analysis to assess factors associated with no HBsAg and HBsAg loss in HBeAg-negative patients (Table 2). Using the ''enter'' method, the results suggested that baseline HBsAg levels were associated with HBsAg loss in patients after follow-up. The baseline HBsAg levels of < 200 IU / mL were considered a risk factor for HBsAg loss.

Baseline HBsAg level as a predictor of HBsAg loss in e antigen-negative CHB infection with PNALT after follow-up

We considered no and HBsAg loss as a categorical variable and baseline HBsAg level as a variable to assess the AUC of baseline HBsAg level in e antigen-negative CHB patients. The AUC of baseline HBsAg level in the e antigen-negative CHB group was 0.772 (95% CI 0.701—0.834, specificity 80.9%, sensitivity 66.7%, cutoff value 426, $P < 0.001$), as shown in Fig. 2.

HBsAg loss associated with different baseline HBsAg level

We divided patients into two groups (≥ 400 IU/L and < 400 IU/L) according to different baseline HBsAg levels. The cumulative probability of HBsAg loss in the HBsAg < 400 IU/L group was 20% (7/35), which was higher than that in the HBsAg ≥ 400 IU/L group (3.88%; 5/129; $X^2 = 11.75$, $P = 0.0006$) (Fig. 3).

Discussion

Elimination of hepatitis B virus is the ultimate goal of treatments for CHB infection. However, the main endpoint of current treatment strategies is the long-term suppression of HBV replication, with HBsAg loss as an optimal endpoint.

The 2017 EASL guidelines recommend that the previously termed ''inactive carriers'' be renamed to e

Table 2 Characteristics of E-negative CHB patients with or (no) HBsAg loss at the baseline.

Factors	No HBsAg loss ($n=152$)	HBsAg loss ($n=12$)	P	Multivariate analysis[c]			
				RR	Wald	95% CI	P
Age	36.83 ± 10.34	41.19 ± 11.15	**0.006**[a]	0.765	0.327	0.153—1.155	0.211
≧40	57 (37.5%)	7 (58.3%)	0.154[b]	1			
<40	95 (62.5%)	5(41.7%)		0.631	0.776	1.043—1.637	0.064
Gender							
man	121 (74.9%)	9 (75%)	0.982[b]	1			
Femal	41 (25.3%)	3 (25%)		0.657	0.088	0.061—1.164	0.844
Vertical transmission of infection history							
Yes	88 (57.9%)	5(41.7%)	0.275[b]	1			
No	64(42.1%)	7(58.3%)		0.925	0.075	0.112—1.147	0.684
BMI	21.43 ± 2.15	22.04 ± 1.27	0.246[a]	1.003	0.643	0.0654—1.754	0.335
HBV DNA (IU /mL)	201.7 ± 101.6	33.4 ± 26.7	**0.003**[a]	1.047	1.322	1.021—2.124	0.121
<2000, ≧200	0 (0%)	0 (0%)	**0.006**[b]	1			
<200,	88 (57.9%)	2 (16.7%)		0.843	0.466	0.384—1.443	0.145
undetectable	64 (42.1%)	10 (83.4%)		1.433	0.198	0.687—1.275	0.086
ALT	24.62 ± 8.43	11.21 ± 6.26	0.33[a]	1.175	0.132	0.14—1.644	0.196
PLT ($\times 10^9$/L)	211.46 ± 52.74	222.53 ± 81.53	0.775[a]	0.055	1.623	1.154—1.754	0.323
LSM (kpa)	5.12 ± 1.71	4.66 ± 1.22	0.06[a]	1.443	0.116	0.684—1.116	0.633
HBsAg (IU /ml)	3334.179 ± 1493.99	1290.163 ± 1019.79	**<0.001**	5.078	7.436	3.655—19.634	**<0.001**
≧2000	31 (21.4%)	2 (16.7%)	**<0.001**[b]	1			
<2000, ≧200	109 (71.7%)	4 (33.3%)		0.843	0.466	0.384—1.443	0.445
<200	12 (7.9%)	6 (50.0%)		6.053	11.445	11.68—211.245	**<0.001**

Parameters are expressed as mean ± SD or number (%) PLT: platelet; ALT: alanine aminotransaferase; BMI: body mass index; LSM: liver stiffness measurement. The normal range of ALT is 5—40 U/L, PLT is 100—300 $\times 10^9$/L. Significance of P values in bold: significantly different between follow-up and baseline patients.

[a] One-way analysis.

[b] Pearson Chi-Square,cFisher exact tests.

[c] Cox regression, method: enter. If methold is stepwise:

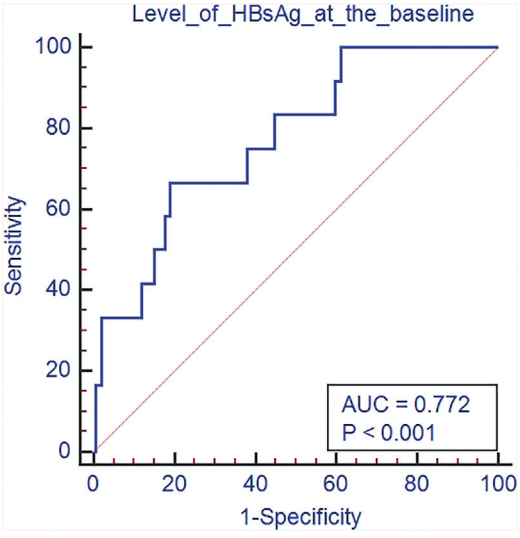

Figure 2 Baseline HBsAg level as a predictor of HBsAg loss in e antigen-negative CHB infection with PNALT after follow-up.

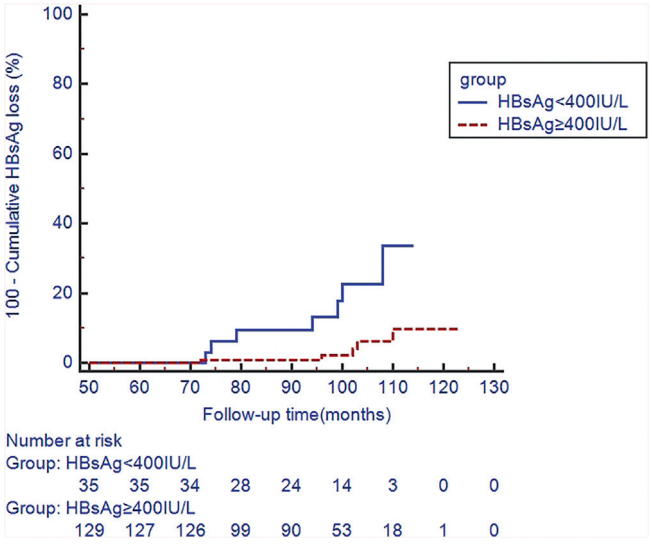

Figure 3 HBsAg loss associated with different baseline HbsAg level.

antigen-negative CHB infection, which is characterized by e antigen-negative low hepatitis B virus load (< 2000 IU/mL) or even undetectable load and PNALT according to traditional cut-off values (ULN 40 IU/mL).

The HBV DNA level is a predictive biomarker that indicates the progression of CHB; thus, HBV viral suppression by antiviral therapy has been shown to improve histology damage, reduce cirrhosis, and even HCC occurrence. During

our follow-up study, it was found that the difference in HBV DNA level was statistically significant ($P < 0.05$) between the baseline and follow-up groups. We observed that 24 patients had HBV DNA level of > 200 IU / mL during the follow-up period. Eight cases of CHB with elevated ALT were associated with HBV DNA level of > 200 IU/mL. Therefore, the level of HBV DNA suppression should be inferred as the lower, the better, although benefits are not well defined (recommend by 2017 EASL guidelines).

LSM performed using a FibroScan is a good noninvasive test for evaluating liver disease severity and prognosis, and our previous studies showed that LSM is superior to the AST-to-platelet ratio index and fibrosis-4 index in PNALT patients due to be influenced by liver inflammation and necrosis [13]. During our follow-up study, LSM were stable in a baseline versus follow-up group owing to the fibrosis recovery in partial patients neutralized the fibrosis progression in some patients.

CHB patients with the characteristics of e antigen-negative chronic HBV infection are considered to have minimal hepatic inflammation and necrosis, and liver fibrosis is not obvious. The risk of progression to cirrhosis or HCC is very low, and these patients have a higher HBsAg loss rate. It has been reported that the annual spontaneous HBsAg loss rate can be 1%—3% [14].Typically, such patients may have low serum HBsAg levels (< 1000 IU/mL) [15]. Our study shown HBsAg loss rate is 0.86% (12/8.5 ys) per year and confirmed that the annual spontaneous HBsAg loss rate is 2.35% (7/35/8.5 ys)in the HBsA < 400 IU/L group was higher than that (0.86%, 5/129/8.5 ys) in the HBsAg ≥ 400 IU/L group.

In different phases of the natural history of HBV virus, serum HBsAg levels are different. HBsAg serum levels are higher in the immunotolerant phase than in the immune-clearance phase (4.5—5 vs. 3.7—4.3 log10/IU/mL) [16,17]. In some Asian reports, high serum HBsAg (> 5 log10/IU/ml) and HBV DNA (> 8 log10/IU/ml) levels were shown to characterize the immunotolerant phase in HBeAg-positive carriers [18—20].

A previous study revealed that in HBeAg-positive patients, HBsAg decline of > 1 log after one year of treatment with telbivudine was predictive for HBsAg loss [21]. Similar findings have also been reported after treatment with tenofovir or entecavir [22,23]. HBsAg decline was due to the activation of the antiviral T cell [24].

The existing evidence indicates that in HBeAg-negative patients, serum HBsAg levels are significantly lower in e antigen-negative CHB infection than in e antigen-negative CHB patients [25]. A large sample study of 1068 cases showed that even with the same HBV DNA viral load (< 20,000 IU/mL), the incidence of CHB in low HBsAg level patients (< 1000 IU/mL) was less than that in high HBsAg level patients (≥ 1000 IU/mL), and the risk of progression to cirrhosis and HCC was also low [26]. In our long-term cohort follow-up, we found three cases of CHB recurrence with HBV DNA recurrence, although it was difficult to confirm recurrence in line with HBsAg levels. It has been suggested that low HBsAg level patients (< 100 IU/mL) have a higher HBsAg loss [27,28]. Our study confirmed that the cumulative probability of HBsAg loss in the HBsAg < 400 IU/L group was higher than that in the HBsAg ≥ 400 IU/L group, indicating that spontaneous HBsAg loss may not occur frequently.

In conclusion, e antigen-negative CHB infection with low HBV DNA level (< 200 IU/mL) and PNALT will progress to chronic hepatitis, although the probability of its occurrence is low. Spontaneous HBsAg loss may not occur frequently because the manifested cumulative probability of HBsAg loss in the HBsAg < 400 IU/L group was higher than that in the HBsAg ≥ 400 IU/L group.

Data statement

We have overviewed the data and all original data can be queried underlying the article.

Disclosure of interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.10.017.

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