HBsAg Spontaneous Seroclearance in a Cohort of HBeAg-Seronegative Patients With Chronic Hepatitis B Virus Infection

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Loss of hepatitis B surface antigen (HBsAg) is considered to reflect the resolution of a hepatitis B virus (HBV) infection. Patient characteristics and various seromarkers were evaluated to characterize factors predicting spontaneous HBsAg loss in a cohort of HBeAg-seronegative patients with presumed chronic HBV infection. Relationships between seromarkers and HBsAg loss were assessed annually and after 6 years using binary logistic regression. Among the 634 participants, 117 (18.45%) cleared HBsAg after 6 years, with a 3.08% annual seroclearance rate. Baseline HBsAg levels and platelet (PLT) counts were predictors of HBsAg seroclearance. The HBsAg level predicted HBsAg seroclearance better than the PLT count (area under the receiver operating characteristic curve (AUROC): HBsAg, 0.965 (95%CI, 0.947–0.980) versus PLT count, 0.617 (95%CI, 0.561–0.669); P < 0.001). A cutoff HBsAg level of 10 IU/ml at baseline predicted spontaneous HBsAg seroclearance at 6 years with a diagnostic accuracy of 93.4%, a sensitivity of 87.2%, a specificity of 94.8%, a positive predictive value of 79.1%, and a negative predictive value of 97.0%. HBsAg seroclearance may occur more commonly than expected. A serum HBsAg level <10 IU/ml and PLT count were accurate predictors of clearance.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem that results in more than one million deaths per year [Chen, 1993]. Patients with chronic HBV infection have an increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) [Kao, 2003; Liaw and Chu, 2009]. It is estimated that among HBV carriers infected with the virus early in life, 25% to 40% will eventually develop these serious complications [Liaw and Chu, 2009]. Despite the increasing availability of effective vaccines, more than 350 million people in the world are chronically infected. The majority of these people reside in the Asia-Pacific region, where infection is usually acquired perinatally or in early childhood [Chen et al., 2000; Custer et al., 2004].

The natural history of a chronic HBV infection acquired in early life involves progression through three phases [Lee, 1997]. The first phase (immune tolerance) features seropositivity for hepatitis B e antigen (HBeAg), a high amount of circulating HBV DNA, and normal levels of alanine aminotransferase (ALT) in the serum. The second phase (immune seroclearance) is characterized by episodic increases in serum ALT levels resulting from specific

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T-lymphocyte-mediated cellular responses to viral antigens and hepatocyte apoptosis. A proportion of patients enter the third and final (residual inactive) phase, which is serologically characterized by low levels of HBV DNA, the absence of HBeAg, the presence of antibodies against HBeAg, and normal ALT levels with the continued presence of hepatitis B surface antigen (HBsAg). Some patients may also enter an HBeAg-negative active chronic hepatitis type B (CHB) phase. This phase is characterized by the absence of HBeAg and high levels of ALT and HBV DNA.

In recent years, the quantification of HBsAg has become an important method for evaluating viral activity. Recent studies have shown that patients in the low replication phase and inactive HBsAg carriers have low serum HBsAg levels of 2.0–2.5 log10 IU/ml [Chan et al., 2010; Jaroszewicz et al., 2010; Nguyen et al., 2010]. A study of patients undergoing antiviral therapy showed that a 2-log10 decrease in HBsAg to a level less than 100 IU/ml was associated with a high likelihood of HBsAg seroclearance [Wiegand et al., 2008]. Another study suggested that HBsAg levels less than 1,000 IU/ml and HBV DNA levels less than 2,000 IU/ml can be used as markers of inactive carriage in patients with HBV genotype D infection [Brunetto et al., 2010]. HBV seromarkers, such as HBV viral load and genotype, are not evaluated during regular health examinations. In contrast, lipids, fasting plasma glucose (GLU), uric acid (UA), and liver-related markers, such as aspartate aminotransferase (AST), ALT, alkaline phosphatase (ALP), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 199, total bilirubin, total protein (TP), albumin (ALB), globulin (GLB), and gamma-glutamyltransferase (GGT), are routinely tested in clinical practice to assess liver injury, inflammation, fibrosis, and dysfunction. This study was conducted to investigate the relationships between these seromarkers and spontaneous HBsAg loss to determine which markers could be helpful for predicting spontaneous HBsAg loss.

MATERIALS AND METHODS

Patients

A total of 12,500 individuals underwent health check-ups at Hua-dong Sanatorium, Wuxi, in 2007 in which serum HBsAg was quantified. Of these individuals, 7,252 (5.8%) HBeAg-seronegative, treatment-naive patients more than 22 years old were enrolled in the current study. These enrolled patients included both HBsAg-inactive carriers and HBeAg-negative active CHB patients. Each enrolled patient signed an informed consent form that was approved by the ethics committee. All the enrollees had been HBsAg-positive for >6 months, and none presented with evidence of infection with hepatitis C virus (HCV), hepatitis D virus (HDV), or human immunodeficiency virus (HIV). Over a follow-up period of 4 ± 1.55 years, 118 patients spontaneously cleared HBsAg. Patients who underwent HBeAg seroreversion (n = 2), developed cirrhosis (n = 14) or HCC (n = 3), received treatments (n = 41), or were lost to follow-up (n = 31) were excluded from the study. The final analysis included 634 participants (87.5% of the enrollees).

Laboratory Methods

All the participants were interviewed in person using a structured questionnaire administered by trained public health doctors. Information on socio-demographic characteristics and lifestyle habits was collected. Using standard sterile techniques, 10-ml overnight fasting blood samples were collected at enrollment and during annual follow-ups. Serum HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc levels were detected using an automated chemiluminescent microparticle immunoassay (Abbott i2000, Abbott Laboratories). A quantitative assay for HBsAg was conducted using an Abbott i2000 with a detection range of 0.05–250 IU/ml. If the HBsAg level was higher than 250 IU/ml, the samples were diluted 1:100 to 1:1,000 to obtain a reading within the range of the calibration curve. An enzyme-linked immunosorbent assay (ELISA) (Wan Tai, Beijing, China) was used as a qualitative method to detect HBsAg with a lowest detectable limit of 0.11 IU/ml. Antibodies for HCV, HDV and HIV were detected with a serum chemistry autoanalyzer (AU5400; Olympus Co., Japan); routine blood markers were also measured using using Wan-Tai assays. Liver function tests as well as blood lipids, GLU, and UA measurements were performed with a serum chemistry autoanalyzer (AU5400; Olympus Co., Japan). Antibodies for HCV, HDV and HIV were detected using a Sysmex 5000 (Sysmex Co., Tokyo, Japan); and AFP, CEA, and CA199 were measured using an automated chemiluminescent microparticle immunoassay (UniCel DXI800; Beckman Coulter Co., Fullerton City, Harbor Blvd.).

Data Collection

At the time of enrollment, the patients were evaluated using an Architect assay for serologic markers (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc). Other serologic markers, including antibodies to HCV, HDV, and HIV as well as liver function parameters, GLU, UA, blood lipids, and routine blood markers were also measured. During the follow-up period, HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc levels were tested annually until HBsAg seroclearance was confirmed by the Architect assay. Other serologic markers were assayed annually regardless of HBsAg clearance. Abdominal ultrasonography was also performed annually using a high-resolution, real-time scanner.

Definition of HBV Infection Status

We classified HBV carriers according to quantitative HBsAg levels at baseline into the following
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Statistical Analysis

Continuous variables are presented as the mean ± SD, and categorical variables are presented as values. Differences between subgroups were analyzed using one-way ANOVA or independent-samples t tests as appropriate for continuous variables. Categorical variables were compared using Crosstabs as appropriate.

Receiver operating characteristic (ROC) curve analysis was used to compute the area under the ROC curve (AUROC). In addition, we evaluated the relationship between several seromarkers and the risk of HBsAg loss by binary logistic regression. Statistical significance was defined as P < 0.05 by two-tailed tests. All the analyses were performed using SPSS software (version 21.0; SPSS, Inc., Chicago, IL) (AAC2010172).

RESULTS

Study Cohort

A total of 634 participants who were HBsAg-seropositive and anti-HCV seronegative and who had no evidence of cirrhosis or HCC were included in the analysis. The baseline data for our patients are shown in Table I. All the participants were divided into three groups: (i) <10 IU/ml; (ii) ≥10 to <200 IU/ml; and (iii) ≥200 IU/ml. HBsAg loss was defined as two consecutive HBsAg levels of <0.05 IU/ml at least 6 months apart. Cirrhosis and HCC were defined by histological or ultrasonographic findings (two consecutive examinations 6 months apart) along with clinical features of splenomegaly, esophageal varices, or ascites [Bruix and Sherman, 2005; Feld et al., 2007].

| TABLE I. Baseline Characteristics of the Study Participants |
|------------------------------------------------------------|
| **HBsAg levels (IU/ml)**                                    |
| Variable                  | <10, n = 129 (20.3%) | <200, n = 143 (22.6%) | ≥200, n = 362 (57.1%) | P-value |
| Gender                   |                        |                        |                        |         |
| Male, n (%)              | 103 (79.8)             | 110 (76.9)             | 272 (75.1)             | 0.714   |
| Female, n (%)            | 26 (20.2)              | 33 (23.1)              | 90 (24.9)              |         |
| Age at enrollment (years)| 51 ± 9                 | 50 ± 9                 | 48 ± 9                 | <0.001  |
| Cigarettesmoker          |                        |                        |                        |         |
| Yes, n (%)               | 79 (61.2)              | 86 (60.1)              | 184 (50.8)             | <0.001  |
| No, n (%)                | 50 (38.8)              | 57 (39.9)              | 178 (49.2)             |         |
| BMI (kg/m²)              | 23 ± 8                 | 24 ± 7                 | 23 ± 9                 | 0.672   |
| Liver function tests     |                        |                        |                        |         |
| ALT (U/L)                | 26 ± 15                | 30 ± 19                | 37 ± 21                | 0.005   |
| AST (U/L)                | 23 ± 9                 | 26 ± 11                | 29 ± 15                | 0.019   |
| GGT (U/L)                | 35 ± 12                | 30 ± 18                | 33 ± 15                | 0.674   |
| ALP (U/L)                | 43 ± 21                | 50 ± 23                | 51 ± 25                | 0.225   |
| TP (g/L)                 | 75.3 ± 4.9             | 76.2 ± 4.1             | 75.5 ± 4.2             | 0.162   |
| ALB (g/L)                | 47.0 ± 2.6             | 47.4 ± 2.2             | 46.9 ± 2.5             | 0.073   |
| GLB (g/L)                | 28.2 ± 3.8             | 28.7 ± 3.1             | 28.7 ± 3.5             | 0.389   |
| ALB/GLB ratio            | 1.7 ± 0.2              | 1.6 ± 0.2              | 1.5 ± 0.2              | 0.339   |
| Total bilirubin (µmol/L) | 14.0 ± 4.4             | 15.0 ± 5.1             | 15.4 ± 6.9             | 0.084   |
| Direct bilirubin (µmol/L)| 3.9 ± 1.6              | 4.1 ± 1.6              | 4.4 ± 3.7              | 0.244   |
| Indirect bilirubin (µmol/L)| 10.1 ± 3.0           | 10.8 ± 3.7             | 10.9 ± 4.0             | 0.116   |
| Blood lipids             |                        |                        |                        |         |
| Triglyceride (mmol/L)    | 1.68 ± 1.87            | 1.56 ± 0.91            | 1.57 ± 1.05            | 0.673   |
| Total cholesterol (mmol/L)| 4.67 ± 0.81          | 4.68 ± 0.68            | 4.70 ± 0.83            | 0.877   |
| HDL cholesterol (mmol/L) | 1.21 ± 1.01            | 1.23 ± 1.20            | 1.21 ± 1.05            | 0.220   |
| GLU (mmol/L)             | 5.54 ± 0.86            | 5.53 ± 0.84            | 5.37 ± 0.86            | 0.066   |
| UA (µmol/L)              | 349 ± 72               | 349 ± 89               | 339 ± 74               | 0.268   |
| Routine blood markers    |                        |                        |                        |         |
| WBC (10⁹/L)              | 6.8 ± 1.8              | 6.6 ± 1.9              | 6.5 ± 1.7              | 0.105   |
| RBC (10¹²/L)             | 4.75 ± 0.40            | 4.69 ± 0.45            | 4.72 ± 0.46            | 0.543   |
| Hemoglobin (g/L)         | 151 ± 13               | 149 ± 17               | 150 ± 15               | 0.565   |
| PLT (10⁹/L)              | 215 ± 54               | 207 ± 47               | 198 ± 53               | 0.006   |
| AFP (ng/ml)              | 3.2 ± 4.3              | 2.7 ± 1.8              | 3.9 ± 3.8              | 0.137   |
| CEA (ng/ml)              | 1.5 ± 0.9              | 1.33 ± 1.0             | 1.1 ± 1.1              | 0.323   |
| CA199 (U/ml)             | 9.8 ± 5.5              | 12.2 ± 6.9             | 13.9 ± 10              | 0.065   |
| HBsAg loss, n (%)        | 102 (79.1)             | 14 (9.8)               | 1 (0.3)                | <0.001  |
| Phase                    |                        |                        |                        |         |
| Inactive carrier, n (%)  | 109 (84.5)             | 116 (81.1)             | 262 (72.4)             | 0.006   |
| Active CHB, n (%)        | 20 (15.5)              | 27 (18.9)              | 100 (27.6)             |         |

The data are presented as the mean ± SD or as the number (percentage). Differences between subgroups were analyzed using one-way ANOVA or independent-samples t tests as appropriate for continuous variables. Categorical variables were compared using Crosstabs.
into three groups according to HBsAg levels at the time of enrollment: <10 IU/ml, 129/634 (20.3%); ≥10 to <200 IU/ml, 143/634 (22.6%); and ≥200 IU/ml, 362/634 (57.1%). Significant differences were noted in age, cigarette smoking, ALT, AST, PLT, and infection phase at baseline. Differences with respect to gender, BMI, GGT, ALP, TP, ALB, GLB, A/G, total bilirubin, direct bilirubin, indirect bilirubin, blood lipids, GLU, UA, WBC, RBC, hemoglobin, AFP, CEA, and CA199 were not statistically significant. At the end of the follow-up period, the HBsAg seroclearance rate was 79.1% (102/129) among participants with baseline HBsAg levels <10 IU/ml, 9.8% among those with baseline HBsAg levels ≥10 to <200 IU/ml (P < 0.001), and 0.3% among those with baseline HBsAg levels ≥200 IU/ml (P < 0.001). A total of 117 of the 634 (18.45%) participants had achieved HBsAg seroclearance at 6 years, with a 3.08% annual and an 18.45% 6-year seroclearance rate. At the end of 2013, 30 patients had lost HBsAg and subsequently gained anti-HBs with a 25.64% (30/117) HBsAg seroconversion rate.

### Baseline Predictors of HBsAg Seroclearance

At the end of the study, participants were divided into two groups according to whether HBsAg seroclearance had occurred: the HBsAg-loss group and the HBsAg-positive group at the end of the study.

| Variable                        | HBsAg loss (17.5%), n = 117 (18.5) | HBsAg-positive (82.5%), n = 517 (81.5) | P-value |
|---------------------------------|------------------------------------|---------------------------------------|---------|
| **Gender**                      |                                    |                                       |         |
| Male, n (%)                     | 93 (79.5)                          | 392 (75.8)                            | 0.257   |
| Female, n (%)                   | 24 (20.5)                          | 125 (24.2)                            |         |
| **Age at enrollment (years)**   | 51 ± 10                            | 48 ± 9                                | <0.001  |
| **Cigarettesmoker**             |                                    |                                       |         |
| Yes, n (%)                      | 67 (57.3)                          | 282 (54.5)                            | 0.799   |
| No, n (%)                       | 50 (42.7)                          | 235 (45.5)                            |         |
| **BMI (kg/m²)**                 | 24 ± 3                             | 25 ± 3                                | 0.727   |
| **Liver function tests**        |                                    |                                       |         |
| ALT (U/L)                       | 27 ± 16                            | 35 ± 39                               | 0.022   |
| AST (U/L)                       | 24 ± 9                             | 28 ± 22                               | 0.071   |
| GGT (U/L)                       | 33 ± 29                            | 32 ± 32                               | 0.853   |
| ALP (U/L)                       | 51 ± 30                            | 55 ± 22                               | 0.119   |
| TP (g/L)                        | 75.2 ± 4.9                         | 75.7 ± 4.2                            | 0.260   |
| ALB (g/L)                       | 46.9 ± 2.7                         | 47.0 ± 2.4                            | 0.451   |
| GLB (g/L)                       | 28.4 ± 3.8                         | 28.7 ± 3.4                            | 0.357   |
| A/G                             | 1.6 ± 0.2                          | 1.7 ± 0.2                             | 0.560   |
| Total bilirubin (µmol/L)        | 14.2 ± 4.8                         | 15.2 ± 6.4                            | 0.095   |
| Direct bilirubin (µmol/L)       | 3.9 ± 1.7                          | 4.3 ± 3.2                             | 0.149   |
| Indirect bilirubin (µmol/L)     | 10.3 ± 3.4                         | 10.8 ± 3.9                            | 0.154   |
| Blood lipids                    |                                    |                                       |         |
| Triglyceride (mmol/L)           | 1.56 ± 0.95                        | 1.60 ± 1.28                           | 0.765   |
| Total cholesterol (mmol/L)      | 4.65 ± 0.78                        | 4.70 ± 0.8                            | 0.576   |
| High density lipoprotein (mmol/L)| 1.28 ± 0.27                      | 1.29 ± 0.32                           | 0.548   |
| GLU (mmol/L)                    | 5.53 ± 0.90                        | 5.42 ± 0.85                           | 0.186   |
| UA (µmol/L)                     | 343 ± 76                           | 344 ± 78                              | 0.869   |
| **Routine blood markers**       |                                    |                                       |         |
| WBC (10⁹/L)                     | 6.9 ± 1.8                          | 6.5 ± 1.7                             | 0.038   |
| RBC (10¹²/L)                    | 4.69 ± 0.38                        | 4.73 ± 0.46                           | 0.419   |
| Hemoglobin (g/L)                | 150 ± 13                           | 150 ± 16                              | 0.999   |
| PLT (10⁹/L)                     | 221 ± 53                           | 200 ± 51                              | <0.001  |
| AFP (ng/ml)                     | 3.0 ± 2.7                          | 3.6 ± 7.0                             | 0.380   |
| CEA (ng/ml)                     | 1.6 ± 0.9                          | 1.4 ± 1.1                             | 0.148   |
| CA199 (U/ml)                    | 9.8 ± 6.6                          | 13.6 ± 22                             | 0.074   |
| **HBsAg levels, n (%)**         |                                    |                                       |         |
| <10 IU/ml                       | 102 (87.1)                         | 27 (5.2)                              | <0.001  |
| ≥10 to <200 IU/ml               | 14 (12.0)                          | 129 (25.0)                            |         |
| ≥200 IU/ml                      | 1 (0.9)                            | 361 (69.8)                            |         |
| **Phase**                       |                                    |                                       |         |
| Inactive carrier, n (%)         | 88 (75.2)                          | 430 (83.2)                            | 0.073   |
| Active CHB, n (%)               | 29 (24.8)                          | 87 (16.8)                             |         |

Italic values indicate P-value <0.05, statistically significant.
The data are presented as the mean ± SD or the number (percentage).
Differences between subgroups were analyzed using one-way ANOVA or independent-samples t tests as appropriate for continuous variables. Categorical variables were compared using Crosstabs.

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the HBsAg-positive group. HBsAg loss was associated with baseline differences in age (P < 0.001), WBC (P = 0.038), PLT count (P < 0.001), ALT activity (P < 0.022), and HBsAg level (P < 0.001) (Table II) but was not associated with gender, cigarette smoking, BMI, AST, GGT, ALP, TP, ALB, GLB, A/G, total bilirubin, direct bilirubin, indirect bilirubin, blood lipids, GLU, UA, RBC, hemoglobin, AFP, CEA, CA199, or infection phase. In the HBsAg-loss group, the mean values for age, WBC, PLT, and ALT at baseline were 51 years, 6.9 × 10^9/L, 221 × 10^9/L, and 27 U/L, respectively. In this group, the baseline HBsAg levels were less than 10 IU/ml in 87.2% of the patients, ≥10 to <200 IU/ml in 12%, and ≥200 IU/ml in 0.9%. Binary logistic regression analysis revealed that HBsAg levels (P < 0.001) and PLT count (P = 0.004) were two statistically significant predictors of HBsAg seroclearance (Table III). Lower HBsAg levels and higher PLT counts were associated with a higher rate of HBsAg loss.

Predicting HBsAg Seroclearance

To compare the predictive ability of HBsAg levels and PLT count, the 6-year predictive accuracy was assessed using ROC curves and the AUROC (Fig. 1A and B). The AUROC significantly increased from 0.617 (95% CI, 0.561–0.669; P < 0.001) for PLT count to 0.965 (95% CI, 0.947–0.980; P < 0.001) for HBsAg levels. The accuracy of HBsAg levels was significantly better than that of PLT count for predicting 6-year HBsAg seroclearance. ROC curve analysis indicated that HBsAg levels remained a significant predictor of 6-year HBsAg seroclearance. With a cutoff HBsAg baseline level of 200 IU/ml, the HBsAg loss rate within 6 years could be predicted with a diagnostic accuracy of 75.2%, a sensitivity of 99.1%, a specificity of 69.8%, a positive predictive value (PPV) of 42.6%, and a negative predictive value (NPV) of 99.7%. With a cutoff baseline HBsAg level of 10 IU/ml, the HBsAg loss rate at 6 years could be predicted with a diagnostic accuracy of 93.4%, a sensitivity of 87.2%, a specificity of 94.8%, a PPV of 79.1%, and an NPV of 97.0%. The results showed that baseline HBsAg levels <10 IU/ml had a higher diagnostic accuracy than those <200 IU/ml.

| Variable             | P       | OR (95%CI)          |
|----------------------|---------|---------------------|
| Age at enrollment    | 0.076   | 1.001 (0.927–1.065) |
| ALT                  | 0.801   | 0.916 (0.953–1.037) |
| WBC                  | 0.741   | 1.042 (0.89–1.248)  |
| PLT                  | 0.004   | 1.013 (1.019–1.029) |
| HBsAg level (IU/ml)  |         |                     |
| <200                 | <0.001  | 43.728 (5.554–331.136) |
| <10                  | <0.001  | 1.525 (201.862–11 837) |

Bold and italic values indicate P-value <0.05, statistically significant.

DISCUSSION

HBsAg seroconversion and HBsAg loss are two milestones that mark the natural history of chronic HBV infection. Spontaneous HBsAg seroconversion occurs in >90% of Asian patients with chronic HBV infection, indicating a transition from the immune clearance phase to the final low replication phase. In addition, HBsAg loss has been considered the best measurable indicator of recovery from HBV infection. In this study, 117 of the 634 (18.45%) chronic HBV carriers spontaneously cleared HBsAg, with an annual seroclearance rate of 3.08%. This rate is substantially higher than previously reported rates of HBsAg seroclearance in high endemic areas, which range from 0.5% to 1.4% [Chu and Liaw, 2007, 2010], indicating that seroclearance may not be as rare as previously thought. However, the higher rate of seroclearance in this cohort compared with other cohorts could be attributed to regular health check-ups. Therefore, the observed HBsAg seroclearance rate may be more representative of a spontaneous event. At the end of the study, we measured HBV DNA in 117 HBsAg loss patients and found that two patients remained positive for HBV DNA. These data implied that HBsAg loss was not completely equivalent to recovery from chronic HBV infection.

The progression of HBV infection is primarily immune-mediated, and HBsAg levels are considered a marker of continued infection in hepatocytes [Kao et al., 2000; Chan et al., 2007; Liaw, 2011]. Recent studies have indicated that a lower HBsAg level can better predict clinical outcomes, especially HBsAg loss [Chan et al., 2011; Chen et al., 2012; Tseng et al., 2012]. Although our study showed that HBsAg seroclearance was associated with baseline age (P < 0.001), WBC (P = 0.038), PLT count (P < 0.001), ALT activity (P = 0.022), and HBsAg level (P < 0.001), further analysis revealed that HBsAg level (P < 0.001) and PLT count (P = 0.004) were two statistically significant predictors of HBsAg seroclearance and that HBsAg level was a more significant predictor than PLT count of the probability of HBsAg seroclearance at 6 years. Moreover, a cut-off HBsAg level of 10 IU/ml, which had better diagnostic accuracy, sensitivity, specificity, PPV, and NPV compared with a cut-off of 200 IU/ml, could better predict subsequent HBsAg loss at 6 years. This finding has implications for practicing physicians intending to determine the odds of HBsAg loss. A lower HBsAg level at baseline likely signifies better host immune control of HBV replication and a reduced concentration of intrahepatic covalently closed circular HBV DNA [Wu et al., 2008; Su et al., 2010].

There were several limitations to our study. First, serum HBV DNA and the genotypes of the participants were not measured. Some studies have shown that low levels of HBV DNA are independent predictors of HBsAg seroclearance [Kwak et al., 2011; Seto...
et al., 2012; Yang et al., 2012; Liu et al., 2013]. Second, because we used quantitative methods to identify HBsAg seropositivity, patients with low HBsAg levels may tend to be over-represented in the HBsAg-positive group. In addition, HBsAg was measured by ELISA during follow-up tests until HBsAg seroclearance was confirmed by the Architect assay. As a result, this study only included baseline HBsAg levels, and long-term changes may require further elucidation. Lastly, this study used abdominal ultrasonography to evaluate cirrhosis and HCC because performing liver biopsies in a community-based study was not practicable. Because ultrasonography is less sensitive than biopsy for diagnosing compensated cirrhosis, the true prevalence and incidence of cirrhosis may have been underestimated.

In conclusion, this study of Chinese patients revealed an annual HBsAg seroclearance rate of 3.08% and a 6-year clearance rate of 18.45%, implying that HBsAg seroclearance is not rare. Although age, ALT and AST activities, PLT count, and HBsAg levels at baseline were associated with spontaneous HBsAg loss, the most important predictors of HBsAg seroclearance were serum HBsAg levels and PLT counts, with HBsAg levels being the most predictive. A cutoff HBsAg level of 10 IU/ml accurately predicted spontaneous HBsAg loss.

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