Int J Clin Exp Med 2018;11(4):3542-3550
www.ijcem.com /ISSN:1940-5901/IJCEM0057872

Original Article

Association between serum levels of hepatitis B e-antigen and severity of liver fibrosis

Min Jing, Min Xu, Runan Zhang, Huiqi Wang, Hanying Liang, Yaping Huang, Genyong Gui, Rong Yang, Shengnan Gong, Jun Fan

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China

Received April 3, 2017; Accepted November 4, 2017; Epub April 15, 2018; Published April 30, 2018

Abstract: The association between serum levels of hepatitis B e-antigen (HBeAg) and the extent of liver fibrosis remains unclear. We investigated this association and sought to identify quantitative biomarkers that might serve as tools for evaluation of liver fibrosis in a total of 249 treatment-naïve chronic hepatitis B (CHB) patients. In all, 163 patients (65%) were HBeAg-positive (HBeAg(+)) and 86 (35%) were HBeAg-negative (HBeAg(-)). The mean levels of hepatitis B virus (HBV) DNA and HBsAg (hepatitis B surface antigen) in HBeAg(+) patients were significantly higher than in HBeAg(-) patients. Serum levels of HBsAg, HBeAg, and HBV DNA were significantly intercorrelated in HBeAg(+) patients. In HBeAg(+) patients, significant fibrosis (stages S2-4) was associated with significantly lower median levels of HBsAg, HBeAg, and HBV DNA than was insignificant fibrosis (stages S0-1). Binary logistic regression analyses showed that the level of qHBeAg (quantitative Hepatitis B e antigen) and the prothrombin time were independent predictors of significant liver fibrosis in HBeAg(+) patients. The area under the receiver operating characteristic (AUROC) curve of levels qHBeAg for predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cut-off value was 2.23 log10 PEIU/mL. For prediction of significant fibrosis, the AUROC curves of HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791), respectively. We found a strong inverse correlation between quantitative HBeAg level and severity of fibrosis in HBeAg(+) patients. HBeAg(+) chronic hepatitis B patients with moderate to severe fibrosis had lower levels of HBeAg, HBsAg, and HBV DNA than those with no or mild fibrosis.

Keywords: Hepatitis B e antigen, liver fibrosis, HBsAg, HBV DNA

Introduction

Hepatitis B virus (HBV) infection is a serious global public health problem. It is estimated that at least 2 billion people are infected with HBV. Of these, ~240 million have chronic hepatitis B (CHB) [1]. Each year, ~650,000 HBV infected persons die of liver failure, cirrhosis, and hepatocellular carcinoma (HCC) caused by HBV infection [2]. HBV does not directly kill liver cells; a pathological immune response is the principal mechanism of liver injury and inflammation. Repair of liver necroinflammation is associated with liver fibrosis [3]. Recurrence of inflammation in CHB patients is important in the development of liver cirrhosis and HCC. It is thus necessary to identify fibrosis early.

Liver biopsy is the reference standard for histological evaluation of liver disease [4], but it is invasive and associated with rare but potentially serious complications [5, 6]. Several noninvasive models, including as the fibrosis score-4, the aspartate transaminase-to-platelet ratio index (APRI), and transient elastography, have been used to predict liver fibrosis. However, such noninvasive tests detect only advanced liver disease [7, 8].

Several researchers have found inverse correlations between serum levels of HBsAg and liver fibrosis stage in hepatitis B e-antigen positive (HBeAg(+)) patients [9-11]. In hepatitis B e-antigen negative (HBeAg(-)) patients, levels of HBV DNA may also correlate with fibrosis severity [12, 13]. Wang found that the serum level of hepatitis B e-antigen served as a novel biomarker for predicting liver fibrosis [14]. A positive correlation was evident between levels of

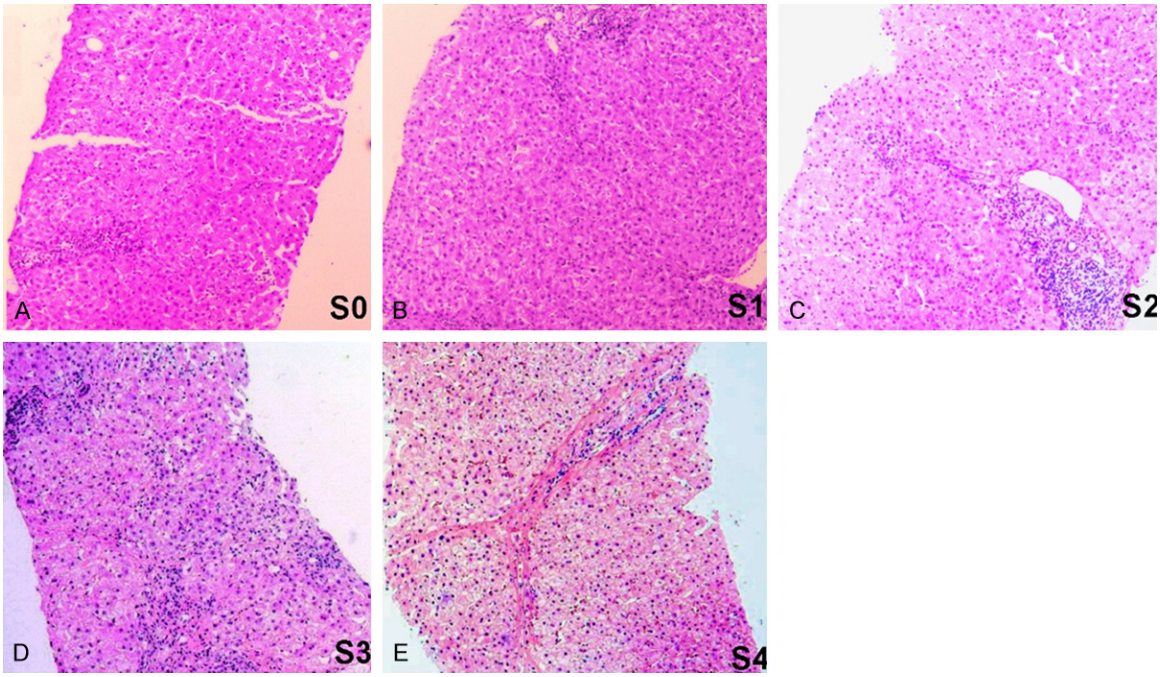

Figure 1. Representative photographs of the stages of liver fibrosis. A. No fibrosis (S0). Photograph of a 40-year-old male patient in the S0 stage. B. Portal fibrosis (S1). Photograph of a 50-year-old male patient in the S1 stage. C. Septum formation (S2). Photograph of a 43-year-old female patient in the S2 stage. D. Architectural distortion (S3). Photograph of a 36-year-old male patient in the S3 stage. E. Cirrhosis, either probable or definite (S4). Photograph of a 48-year-old female patient in the S4 stage.

HBeAg and HBsAg and a strong correlation between levels of HBeAg and HBV DNA [14-16]. Lower extents of immune-mediated liver injury were associated with higher levels of HBeAg [17]. However, the relationship between levels of HBeAg and liver fibrosis remains unclear. Therefore, we investigated the association between HBeAg levels and liver fibrosis and sought quantitative biomarkers that might serve as tools for the evaluation of liver fibrosis in treatment-naïve CHB patients.

Materials and methods

Patients

A total of 249 asymptomatic treatment-naïve patients with chronic HBV infections who attended the Department of Infectious Diseases, the First Affiliated Hospital, Zhejiang University, between January 2012 and October 2016, were prospectively enrolled. The inclusion criteria were: (1) HBsAg(+) status for more than 6 months; (2) absence of HCV, HDV, or HIV co-infection, autoimmune hepatitis, alcoholic liver disease, and drug-induced liver disease; (3) the absence of decompensated liver cirrhosis and HCC; and, (4) the absence of malignancy.

Laboratory measurements

Serum samples and liver biopsies were obtained on the same day. Complete blood cell counts and biochemical test data, including levels of alanine transaminase (ALT), (aspartate transaminase) AST, albumin, cholinesterase, and total bilirubin, and prothrombin time were obtained using automated techniques. Serum levels of HBsAg and HBeAg were quantified using the Architect assay (Abbott Laboratories, Abbott Park, Chicago, IL, USA). The linear range of the HBsAg assay was 0.05-250 IU/mL. Samples were serially diluted (to 1:500) if the HBsAg levels were >250 IU/mL. The hepatitis B e-antigen titers were directly proportional to the chemiluminescence emitted, measured in relative light units (RLUs). The HBeAg levels were expressed as the ratios of the sample RLUs to the control cut-off RLU (S/CO). An HBeAg titer >1.0 S/CO was considered positive. The Paul Ehrlich Institute (PEI) HBeAg reference standard was used to create the standard curve, and the S/CO values were converted into PEIU/mL [18]. Serum levels of HBV DNA were

Table 1. Association between liver fibrosis and clinical parameters

Variables	S0-1	S2	S3	S4
Sex (Male:Female)	74/24	44/31	27/16	21/7
Age (years)	36.17±9.40	36.53±9.63	35.79±8.60	41.24±9.86[a,b]
HBV DNA (log10 IU/ml)	6.72±2.03	6.48±1.83	5.93±1.70[a]	5.72±1.71[a]
HBsAg (log10 IU/ml)	3.82±0.99	3.70±0.76	3.47±0.59	3.17±0.35[a,b]
Anti-HBs (mIU/ml)	0.69±1.40	1.65±4.04	0.79±2.11	0.44±0.68
HBeAg (log10 PEIU/ml)	2.04±0.85	1.06±1.09	0.77±1.06[a]	0.68±0.78[a]
ALT (U/L)	65.08±69.58	74.91±85.00	67.18±76.91	67.14±66.03
AST (U/L)	41.44±39.36	45.40±31.70	54.02±51.72	45.68±32.29
ALB (g/L)	45.42±4.78	44.79±3.48	43.40±5.56	43.71±5.24
CHE (U/L)	8583.01±1895.249	7821.95±2060.32[a]	6618.70±1488.98[a,b]	7075.32±2290.60[a]
TBIL (μmol/L)	14.16±7.83	13.67±7.07	14.51±8.27	14.89±7.43
PT (s)	11.41±0.72	11.82±0.75[a]	12.14±1.04[a]	12.32±1.34[a]
PLT (10E9/L)	188.45±44.24	172.73±46.67	156.28±46.37	142.14±71.08
APRI	0.95±1.15	1.09±1.08	1.10±1.04	1.36±1.25
FIB-4	1.12±0.99	1.31±0.92	1.53±0.76	2.14±1.56[a,b]

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; Anti-HBs, Hepatitis B surface antibody; HBeAg, hepatitis E antigen; ALT, alanine transaminase; AST, aspartate transaminase; ALB, albumin; CHE, Cholinesterase; TBIL, Total Bilirubin; PT, Prothrombin time; PLT, Platelets; FIB-4, Fibrosis-4; APRI, AST to Platelet Ratio Index; [a]compared with S0-1, $P < 0.05$; [b]compared with S2, $P < 0.05$. According to the Scheuer score, liver fibrosis was divided into stages S0-S4. The age, HBV DNA, HBsAg, HBeAg, cholinesterase, prothrombin time and fibrosis-4 were significantly different among the groups. There was no significant difference in the grade or frequency of anti-HBs, ALT, AST, APRI, platelets, total bilirubin, or albumin levels among liver-fibrosis stages. The significance of differences between different stages of liver fibrosis was explored using Pearson's chi-square test for qualitative data and the t-test and analysis of variance for quantitative data.

quantified by real-time fluorescence quantitative polymerase chain reaction on an SLAN-96S platform; the lower limit of detection was 1,000 IU/mL.

Liver biopsy

Percutaneous liver biopsy was performed under ultrasonographic guidance using a 16-gauge needle to obtain a biopsy sample ≥1.5 cm in length with at least six portal tracts. The specimens were fixed, paraffin-embedded, sectioned continuously, and stained with hematoxylin and eosin, Masson's trichrome, and reticulin. Liver fibrosis was staged using the Scheuer classification [19]: no fibrosis (S0); portal fibrosis (S1); septum formation (S2); architectural distortion (S3); and cirrhosis, either probable or definite (S4). Liver biopsy is the gold standard for diagnosis of liver fibrosis. The degree of hepatic fibrosis is divided into stages S0-S4, as recommended by the Chinese Medical Association Hepatology Society in 2000 [20]. In this study, fibrosis of Scheuer grade < 2 (S0-1) was considered insignificant. Significant fibrosis reflected spread beyond the portal tract (S2-4). Representative photographs of the stages of liver fibrosis in 249 patients are shown in **Figure 1**.

Data analysis

Continuous variables are expressed as means with SDs. The levels of HBeAg, HBsAg, and HBV DNA were logarithmically transformed. The significance of differences between different stages of liver fibrosis was explored using Pearson's chi-square test for qualitative data and the t-test and analysis of variance for quantitative data. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test. Binary logistic regression analyses identified factors independently associated with significant liver fibrosis. An area under the receiver operating characteristic (AUROC) curve was used to determine the cut-off values for serum HBV markers predicting significant liver fibrosis. All tests were two-sided and *P* values < 0.05 were considered to reflect statistical significance. All statistical analyses were performed with the aid of SPSS software ver. 20.0 (SPSS, Chicago, IL, USA).

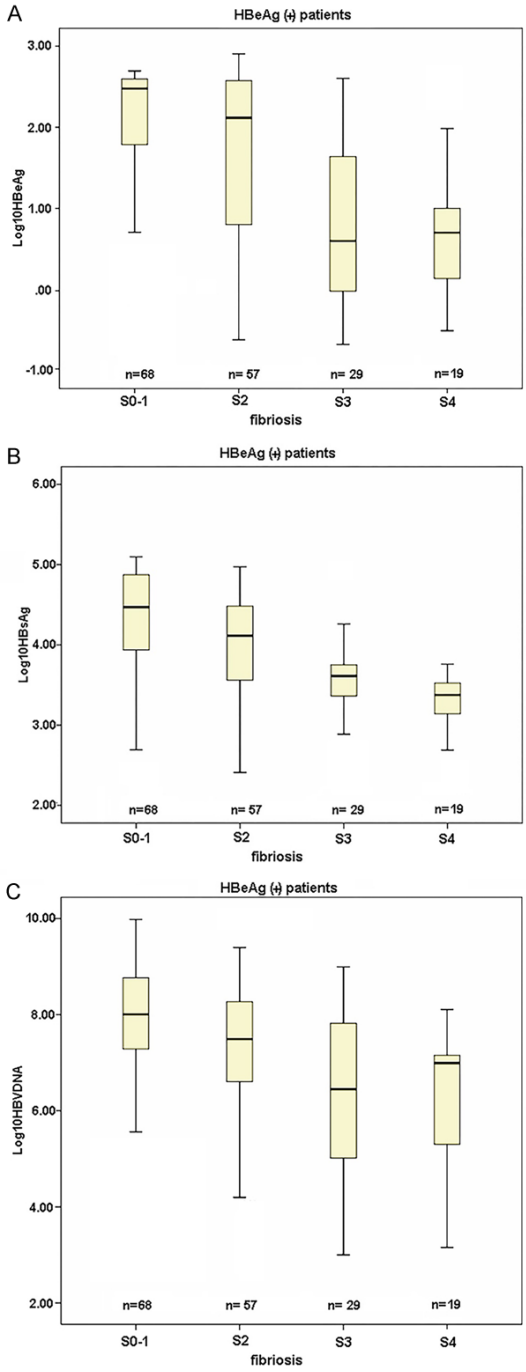

Figure 2. Associations between the serum levels of HBV markers and fibrosis stage in HBeAg(+) patients. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test. A. Serum HBeAg levels are significantly correlated with fibrosis severity in HBeAg(+) patients (r = -0.241, $P < 0.001$). B. Correlation between HBsAg levels and fibrosis severity in HBeAg(+) patients. As fibrosis becomes aggravated, the HBsAg levels trend downward. A strong correlation is evident between the serum HBsAg level and fibrosis severity (r = -0.506, $P < 0.001$). C. An inverse correlation is evident between the HBV DNA level and fibrosis severity in HBeAg(+) patients (r = -0.384, $P < 0.001$).

Results

Patient characteristics

We studied 249 treatment-naïve CHB patients, of whom 163 (65%) were HBeAg(+) and 86 (35%) HBeAg(-). There were 171 (69%) males and 78 (31%) females aged 18-63 years old (mean, 36.78±9.47 years old). The mean levels of HBV DNA and HBsAg were 7.20 and 3.99 log10 IU/mL, respectively, in HBeAg(+) patients, significantly higher than in HBeAg(-) patients. In HBeAg(+) patients, the mean HBeAg level was 1.55 PEIU/mL. Compared to patients with insignificant fibrosis, those with significant fibrosis had lower levels of HBsAg, HBeAg, and HBV DNA. We found no significant difference in fibrosis grade or the level of anti-HBs, ALT, AST, APRI, platelets, total bilirubin, or albumin among patients with different grades of liver fibrosis. However, age, prothrombin time, grade 4 fibrosis, and the levels of HBV DNA, HBsAg, HBeAg, and cholinesterase differed significantly between the groups (all $P < 0.05$) (**Table 1**).

Correlations among serum levels of HBsAg, HBeAg, and HBV DNA

Levels of HBsAg and HBeAg were significantly correlated in HBeAg(+) patients (r = 0.572, $P < 0.0001$) as were levels of HBV DNA and HBeAg (r = 0.603, $P < 0.0001$) and levels of HBsAg and HBV DNA (r = 0.623, $P < 0.0001$). No significant correlation between HBsAg and HBeAg levels or HBVDNA and HBeAg levels was apparent in HBeAg(-) patients. The correlation between HBsAg and HBV DNA levels in HBeAg(+) patients was significantly higher than that in HBeAg(-) patients (r = 0.623, $P < 0.001$ and r = 0.226, $P < 0.05$, respectively).

Associations between the levels of serum HBV markers and fibrosis stage

As fibrosis became aggravated, levels of HBsAg, HBeAg, and HBV DNA in HBeAg(+) patients trended downward (**Figure 2A-C**). Levels of HBsAg, HBeAg, and HBV DNA were negatively correlated with the extent of liver fibrosis in HBeAg(+) patients (r = -0.506, $P < 0.001$; r = -0.241, $P < 0.0001$; r = -0.384, $P < 0.0001$, respectively). The level of HBsAg showed a stronger inverse correlation with liver fibrosis than did the levels of HBeAg and HBV DNA. However, we found no significant correlation between the levels of HBsAg, HBeAg, or HBV

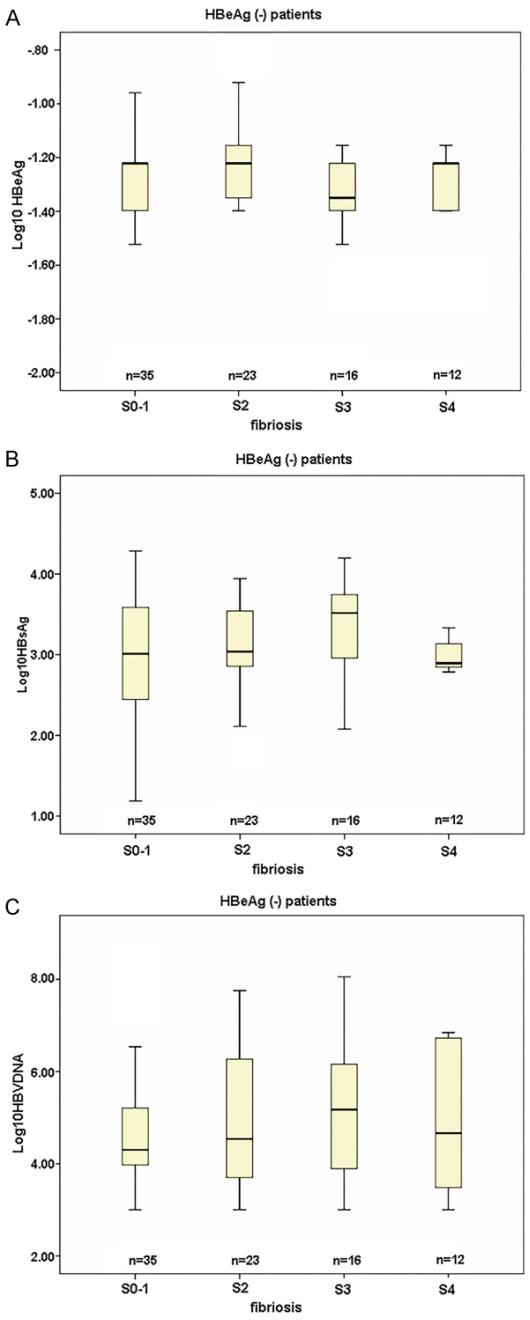

Figure 3. Associations between the levels of serum HBV markers and fibrosis stage in HBeAg(-) patients. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test. A. No correlation is evident between the HBeAg level and fibrosis severity in HBeAg(-) patients. B. No correlation is evident between the HBsAg level and fibrosis severity in HBeAg(-) patients. C. No correlation is evident between the HBV DNA level and fibrosis severity in HBeAg(-) patients.

DNA and the severity of fibrosis in HBeAg(-) patients (**Figure 3A-C**).

HBV marker levels identify significant fibrosis

We stratified patients into two groups on the basis of the extent of fibrosis, and calculated the mean levels of HBV markers (**Table 2**). HBeAg(+) patients with significant fibrosis (stages S2-4) had significantly lower median levels of HBsAg, HBeAg, and HBV DNA than did those with insignificant fibrosis (stages S0-1). However, we found no significant correlation between levels of HBsAg, HBeAg, and HBV DNA and severity of fibrosis in HBeAg(-) patients.

Logistic regression modeling via ROC analysis

HBeAg(+) patients were divided into two groups in terms of the extent of fibrosis: S0-1 and S2-4. Binary logistic regression analyses showed that the HBeAg level and the prothrombin time were independent predictors of significant liver fibrosis. We drew ROC curves to seek to identify significant fibrosis on the basis of the levels of HBsAg, HBeAg, and HBV DNA. The AUROC curve of the level of HBeAg predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cutoff was 2.23 log10 PEIU/mL, with a sensitivity of 72.5% and a specificity of 67.3%. The AUROC curves of HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791). The HBsAg cut-off value was 4.15 log10 IU/mL. This predicted significant fibrosis with a sensitivity of 73.6% and a specificity of 70.8%. An HBV DNA cutoff of 7.74 log10 IU/mL predicted significant fibrosis with a sensitivity of 71.4% and a specificity of 61.5% (**Figure 4**).

Discussion

The annual incidence of cirrhosis in patients with CHB is 2-10% [21]. It is necessary to intervene as early as possible (before cirrhosis develops) to slow the progression of the disease. The 2012 clinical practice guidelines of the European Association for the Study of the Liver and Chinese Medical Association Hepatology Society recommend that patients with moderate to severe fibrosis (S2-4) should receive antiviral treatment [22, 23]. Therefore, it is essential to identify fibrosis early.

HBeAg(+) patients had higher mean HBsAg, HBeAg, and HBV DNA levels than HBeAg(-) patients. Levels of HBsAg and HBV DNA were significantly correlated. Moreover, the HBeAg level was significantly correlated with the

Table 2. Serum levels of HBV markers according to HBeAg status and fibrosis stage

Fibrosis score*	All (n = 249)	HBeAg(+) (n = 163)	HBeAg(-) (n = 86)
Mean HBsAg level log10 IU/ml			
S0-S1	3.80±0.98 (n = 103)	4.24±0.79 (n = 68)	2.96±0.74 (n = 35)
S2-S4	3.54±0.67 (n = 146)	3.74±0.64 (n = 95)	3.13±0.56 (n = 51)
P value	0.017	< 0.001	0.211
Mean Serum HBV DNA log10 IU/ml			
S0-S1	6.71±2.02 (n = 103)	7.76±1.50 (n = 68)	4.71±1.20 (n = 35)
S2-S4	6.18±1.79 (n = 146)	6.80±1.64 (n = 95)	4.98±1.47 (n = 51)
P value	0.033	< 0.001	0.345
Mean Serum HBeAg log10 PEIU/ml			
S0-S1	0.91±1.75 (n = 103)	2.04±0.86 (n = 68)	-1.33±0.04 (n = 35)
S2-S4	0.38±1.52 (n = 146)	1.23±1.12 (n = 95)	-1.31±0.04 (n = 51)
P value	0.013	< 0.001	0.57

*According to the Scheuer score. In HBeAg(+) CHB patients, lower serum HBeAg, HBsAg and HBV DNA levels were more frequent in those with moderate-to-severe fibrosis (stages S2-S4) than patients with no or mild fibrosis (stages S0-S1). There was no significant correlation between HBsAg, HBeAg, and HBV DNA levels with the severity of fibrosis in HBeAg(-) patients. Correlations between Serum levels of HBV markers and fibrosis stage were analyzed using the Spearman correlation test.

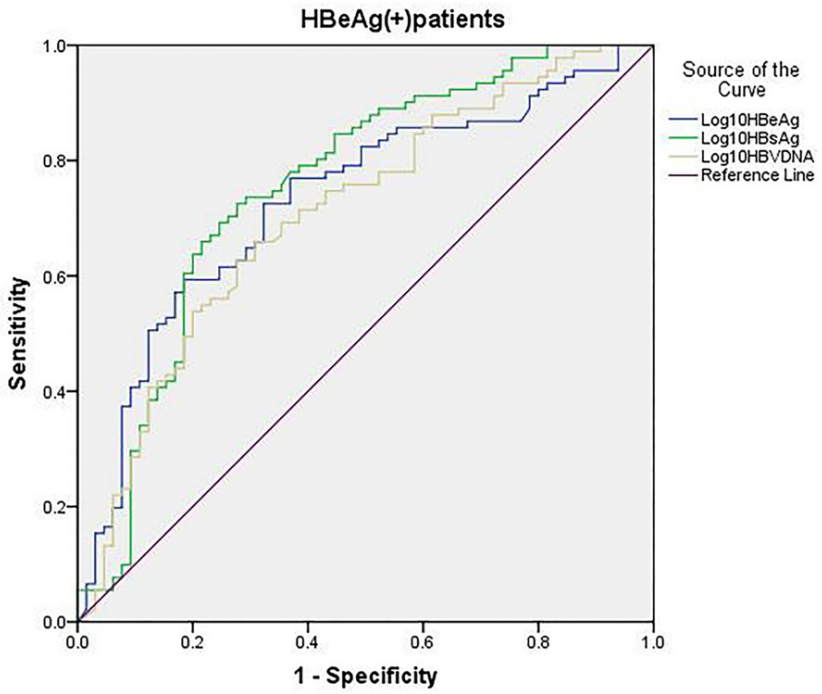

Figure 4. The diagnostic performance of HBV serum markers used to identify significant liver fibrosis in HBeAg(+) CHB patients. Binary logistic regression analyses identified factors independently associated with significant liver fibrosis. The AUROC curve of the serum HBeAg level predicting significant fibrosis was 0.732 (95% CI 0.651-0.812). The cut-off was 2.23 log10 PEIU/mL, with a sensitivity of 72.5% and a specificity of 67.3%. The AUROC curves of the serum HBsAg and HBV DNA levels were 0.755 (95% CI 0.674-0.835) and 0.708 (95% CI 0.626-0.791). The HBsAg cut-off value was 4.15 log10 IU/mL. This predicted significant fibrosis with a sensitivity of 73.6% and a specificity of 70.8%. An HBV DNA cut-off of 7.74 log10 IU/mL predicted significant fibrosis with a sensitivity of 71.4% and a specificity of 61.5%.

HBsAg level in HBeAg(+) patients, as were the HBV DNA and HBeAg levels, consistent with previous reports [14, 15, 24]. The natural history of HBV infection involves interactions between the virus, the host, and the environment [25, 26]. HBV does not directly kill liver cells; a pathological immune response is the principal cause of liver injury and inflammation. HBeAg inhibits the nonspecific immune response by interfering with the antiviral signaling pathways activated by the Toll-like receptors and the product of the retinoic acid-inducible gene I (RIG-I). Thus, HBV replication may be enhanced. High e-antigen concentrations may suppress the immune response. Immune tolerance develops; HBV DNA is vigorously replicated; and the HBsAg levels rise. In HBeAg(-) patients, the levels of HBsAg, HBeAg, and HBV DNA were not intercorrelated.

We found that serum HBV marker levels varied in patients with different stages of fibrosis. Compared to those with insignificant fibrosis, HBeAg(+) patients with significant fibrosis had significantly lower levels of HBsAg, HBeAg, and HBV DNA, as previously described [9, 27]; the reason is unclear. The immune response differs during progression from the immune toler-

ance to the immune clearance phase. During immune clearance, the immune response is strong, and the liver may exhibit moderate to severe inflammation and necrosis, with rapid development of fibrosis. It is possible that liver injury becomes more serious as fibrosis progresses. At this time, the synthesis and release of HBsAg and HBeAg are reduced and HBV DNA replication decreases.

Unconditional logistic regression showed that the HBeAg level and the prothrombin time were independent predictors of significant liver fibrosis in HBeAg(+) patients. The prothrombin time reflects liver coagulation function, and is increases as liver injury progresses. The serum HBeAg level plays an important role in the identification of treatment-naïve HBeAg(+) CHB patients with significant fibrosis. Although HBeAg is not essential for viral assembly or replication, it is important during natural infection in vivo. Higher serum HBeAg levels may reflect impaired immune status. As the HBeAg level falls, the immune response may increase to induce liver fibrosis. The AUROC curves showed that serum HBeAg, HBsAg, and HBV DNA levels of 0.732, 0.755, and 0.708, respectively, predicted significant fibrosis in HBeAg(+) patients.

Our study had several limitations. We enrolled only Chinese patients and did not assay the HBV genotype or the basal core promoter (BCP)/precore (PC) sequence thought to be associated with liver histological activity and fibrosis [9, 15, 27]. These tests are not routine in our hospital. Repeat inflammation is significant in terms of progression of CHB to cirrhosis and even HCC. Liver biopsy is invasive, but long-term monitoring of serum markers can be used to monitor disease progression.

In conclusion, we found a strong inverse correlation between serum levels of HBeAg and fibrosis severity in HBeAg(+) patients. Patients with moderate to severe fibrosis had lower HBeAg, HBsAg, and HBV DNA levels than did those with no or mild fibrosis.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (30872-239) and Zhejiang provincial natural science foundation of China (LY14H190002).

Disclosure of conflict of interest

None.

Address correspondence to: Jun Fan, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, China. Tel: + 86 571 87236581; Fax: +86 571 87236444; E-mail: fanjun@zju.edu.cn

References

[1] Ott JJ, Stevens GA, Groeger J and Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012; 30: 2212-2219.

[2] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D,

Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. Lancet 2012; 380: 2095-2128.

[3] Chu CM. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 2000; 15 Suppl: E25-30.

[4] Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM and Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016; 63: 261-283.

[5] McGill DB, Rakela J, Zinsmeister AR and Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. Gastroenterology 1990; 99: 1396-1400.

[6] Bedossa P and Carrat F. Liver biopsy: the best, not the gold standard. J Hepatol 2009; 50: 1-3.

[7] Xiao G, Yang J and Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology 2015; 61: 292-302.

[8] Sanai FM, Babatin MA, Bzeizi KI, Alsohaibani F, Al-Hamoudi W, Alsaad KO, Al Mana H, Handoo FA, Al-Ashgar H, Alghamdi H, Ibrahim A, Aljumah A, Alalwan A, Altraif IH, Al-Hussaini H, Myers RP and Abdo AA. Accuracy of international guidelines for identifying significant fibrosis in hepatitis B e antigen--negative patients with chronic hepatitis. Clin Gastroenterol Hepatol 2013; 11: 1493-1499, e1492.

[9] Goyal SK, Jain AK, Dixit VK, Shukla SK, Kumar M, Ghosh J, Ranjan A, Gupta N and Tripathi M. HBsAg Level as Predictor of Liver Fibrosis in HBeAg Positive Patients With Chronic Hepatitis B Virus Infection. J Clin Exp Hepatol 2015; 5: 213-220.

[10] Seto WK, Wong DK, Fung J, Ip PP, Yuen JC, Hung IF, Lai CL and Yuen MF. High hepatitis B surface antigen levels predict insignificant fibrosis in hepatitis B e antigen positive chronic hepatitis B. PLoS One 2012; 7: e43087.

[11] Liang LB, Zhu X, Yan LB, Du LY, Liu C, Chen LY, Liao J and Tang H. Serum hepatitis B surface antigen titer and transient elastography in screening for insignificant fibrosis in HBeAg-positive chronic hepatitis B patients. Ther Clin Risk Manag 2015; 11: 229-235.

[12] Bai H, Liu H, Chen X, Xu C and Dou X. Influence of age and HBeAg status on the correlation between HBV DNA and hepatic inflammation and fibrosis in chronic hepatitis B patients. Dig Dis Sci 2013; 58: 1355-1362.

[13] Papatheodoridis GV, Manesis EK, Manolakopoulos S, Elefsiniotis IS, Goulis J, Giannousis J, Bilalis A, Kafiri G, Tzourmakliotis D and Archimandritis AJ. Is there a meaningful serum hepatitis B virus DNA cutoff level for therapeutic decisions in hepatitis B e antigen-negative chronic hepatitis B virus infection? Hepatology 2008; 48: 1451-1459.

[14] Wang H, Yan R, Zhou Y, Wang MS, Ruo GQ and Cheng MJ. Comparison of hepatitis B surface antigen and e antigen in predicting liver histology in hepatitis B e antigen-positive chronic hepatitis B patients. Hepatol Int 2014; 8: 216-223.

[15] Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, Slavin J, Bowden S, Gane EJ, Abbott W, Lau GK, Lewin SR, Visvanathan K, Desmond PV and Locarnini SA. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. Hepatology 2010; 51: 1933-1944.

[16] Demiroren K, Kocamaz H and Dogan Y. The importance of the serum quantitative levels of hepatitis B surface antigen and hepatitis B e antigen in children with chronic hepatitis B. Turk J Gastroenterol 2015; 26: 36-41.

[17] Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, Wong BC, Lai KC and Lai CL. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. Gut 2005; 54: 1610-1614.

[18] Wursthorn K, Zacher BJ, Jaroszewicz J, Darnedde M, Manns M and Wedemeyer H. Development of a protocol for the quantitative determination of HBeAg using the Elecsys(R) HBeAg immunoassay. J Viral Hepat 2011; 18: e179-183.

[19] Scheuer PJ, Standish RA and Dhillon AP. Scoring of chronic hepatitis. Clin Liver Dis 2002; 6: 335-347, v-vi.

[20] Prevention and treatment of viral hepatitis. Chinese Journal Of Hepatology 2000; 8: 324.

[21] Fattovich G, Bortolotti F and Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008; 48: 335-352.

[22] EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012; 57: 167-185.
[23] Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association, Hou JL, lai W. [The guideline of prevention and treatment for chronic hepatitis B: a 2015 update]. Zhonghua Gan Zang Bing Za Zhi 2015; 23: 888-905.
[24] Deguchi M, Yamashita N, Kagita M, Asari S, Iwatani Y, Tsuchida T, Iinuma K and Mushahwar IK. Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. J Virol Methods 2004; 115: 217-222.
[25] Yim HJ and Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. Hepatology 2006; 43 Suppl 1: S173-181.
[26] Stoop JN, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG and Janssen HL. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology 2005; 41: 771-778.
[27] Martinot-Peignoux M, Carvalho-Filho R, Lapalus M, Netto-Cardoso AC, Lada O, Batrla R, Krause F, Asselah T and Marcellin P. Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naive, e antigen-positive patients. J Hepatol 2013; 58: 1089-1095.