Original Article

Establishment and validation of a diagnostic nomogram for significant histopathologic changes of hepatic injury in HBV-infected patients

Lin-Lin Sun[1,2], Min Wang[3], Nan-Nan Zhang[4], Ming-Xia Chen[3], Yuan-Yuan Li[3], Shuai Zhang[5], Cheng-Yong Qin[1]

[1]Department of Gastroenterology, Shandong Provincial Hospital, Shandong University, Jinan, China; [2]Department of Hepatology, Yantai Qishan Hospital, Yantai, China; [3]Department of Pathology, Yantai Qishan Hospital, Yantai, China; [4]Department of Hepatology, Zaozhuang Central Hospital of Shandong Healthcare Group, Zaozhuang, China; [5]Department of Laboratory, Yantai Qishan Hospital, Yantai, China

Contributions: (I) Conception and design: LL Sun, CY Qin; (II) Administrative support: CY Qin; (III) Provision of study materials or patients: LL Sun, M Wang, MX Chen, YY Li, S Zhang; (IV) Collection and assembly of data: LL Sun, NN Zhang; (V) Data analysis and interpretation: LL Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Cheng-Yong Qin. Department of Gastroenterology, Shandong Provincial Hospital, Shandong University, 324 Jingwu Road, Jinan 250021, China. Email: chengyong_qin@126.com.

Background: Significant histopathologic changes of hepatic injury (SHCHI) play a decisive role in evaluating the condition and initiating antiviral in hepatitis B virus (HBV)-infected patients, especially those with normal or mildly elevated alanine transaminase levels. Considering that non-invasive methods were established through experience with chronic hepatitis C, the aim of this study was to establish and verify a nomogram based on hepatitis B for diagnosing SHCHI.

Methods: Three hundred eighty-four patients who fulfilled requirements for participation were randomly assigned to training cohort (n=270) and validation cohort (n=114) according to 7:3. The selection criteria for clinical factors were based on the previous research papers. SHCHI was subgrouped as followed: grade ≥ G2 inflammation and/or stage ≥ S2 fibrosis. The predictive accuracy and discriminative ability of nomogram were determined by a concordance index (C-index), calibration curve and the area under the receiver-operating characteristic curve (AUROC). We also compared diagnostic value of nomogram with model for AST-to-PLT ratio index (APRI) score and model for Fibrosis-4 (FIB-4) score.

Results: Two hundred and two patients (74.44%) and 87 patients (76.32%) were diagnosed as SHCHI, in the training and validation cohort. Logistic regression analysis illustrated that hepatitis B e antigen (HBeAg), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), and prothrombin time (PT) all independently served as risk factors for SHCHI (P<0.05) and were thus utilized to create the nomogram. The nomogram had well-fitted calibration curves and attained excellent concordance indices of 0.80 and 0.75. The sensitivity of nomogram in the diagnosis of SHCHI was 79.7%, the specificity was 68.1%. The area under the curve {AUC; 0.80 [95% confidence interval (CI): 0.74–0.86]} for diagnosing SHCHI by the nomogram was greater in comparison to that of APRI [0.78 (95% CI: 0.71–0.84)], and FIB-4 [0.76 (95% CI: 0.69–0.82)]. Patients with nomogram scores less than 119 were considered to have a lower risk of SHCHI.

Conclusions: The constructed nomogram is suitable to serve as a SHCHI screening tool in chronic HBV-infected patients. But the dependability of the nomogram will necessitate further confirmation in a prospective study and further external validation is needed.

Keywords: Nomogram; non-invasive model; significant histopathologic changes of hepatic injury (SHCHI); chronic hepatitis B; normal or mildly elevated ALT

Submitted Oct 31, 2022. Accepted for publication Dec 29, 2022. Published online Jan 09, 2023.
doi: 10.21037/atm-22-5840
View this article at: https://dx.doi.org/10.21037/atm-22-5840

Introduction

Indicators for therapy in patients with chronic hepatitis B virus (HBV) infection include viremia and the severity of liver fibrosis and inflammation (1,2). As liver inflammation can only be determined by the use of a liver biopsy, serum alanine transaminase (ALT) levels are often employed for risk stratification (3). However, several recent studies have suggested that patients whose levels of ALT are considered "normal" may be at risk for liver histological alteration and may even require antiviral treatment (4-6). Since using ALT and HBV DNA levels without employing a liver biopsy to identify an "inactive carrier status" could miss histologically relevant illnesses in a proportion of patients (7), the recent recommendations from the American Association for the Study of Liver Disease (AASLD) advise that patients with increased HBV DNA levels who have no therapeutic indication based on (non-invasively measured) fibrosis stage should undergo a liver biopsy to rule out severe inflammatory responses (2). Hence, the most reliable approach for examining the stage of liver fibrosis and thus the most appropriate therapeutic choice is the liver biopsy (8). However, because of the high cost and the invasive nature of the surgery, which could increase patient discomfort and the risk of bleeding and pneumothorax, its usage is restricted (9). Considering that chronic HBV infection may cause a wide variety of illnesses and has such a complex natural history, the World Health Organization (WHO) guidelines (10) proposed that in addition to the clinical criteria and the other laboratory parameters (HBV DNA and ALT levels), the non-invasive fibrosis models should be employed.

To date, liver fibrosis has inspired the development of more than 30 different non-invasive diagnostic methods, of which the aspartate aminotransferase (AST) to platelet (PLT) ratio index (APRI), Fibro test, and the FIB-4 index, red cell distribution width to PLT ratio (RPR), 2-dimensional shear wave elastography (2D-SWE), γ-glutamyl transpeptidase to PLT ratio (GPR), point shear wave elastography (P-SWE), transient elastography (TE), as well as diffusion-weighted magnetic resonance in predicting liver fibrosis in patients with hepatitis B and C infections, have higher value (11,12). However, the majority of these methods were established through experience with chronic hepatitis C, and the WHO has only approved APRI, FIB-4, and FibroScan as alternatives for liver biopsy in chronic HBV infection (10). However, in CHB patients, the performance of FIB-4 and APRI in distinguishing between fibrosis stages has not been well established (10). The study demonstrated (10) that APRI and FIB-4 non-invasive models did not show distinct advantage over ALT to predict significant inflammation (G ≥2) in the HBV-infected patients with normal or mildly elevated ALT levels. The lack of simple and convenient evaluations remains a major problem in clinical practice especially in the patients with normal or mildly elevated ALT levels. Consequently, a more finely-tuned approach for predicting personalized liver fibrosis and inflammatory responses of chronic HBV-infected patients is necessary. In recent years, nomograms have been emphasized in the prediction of HBV-related diseases, such as, in predicting survival in patients with acute-on-chronic HBV liver failure after liver transplantation (13), in assessing mortality in patients with acute and chronic HBV liver failure (14).

The present study aimed to create and verify a nomogram for detecting significant histopathologic changes in hepatic injury (SHCHI) in chronic HBV-infected patients exhibiting normal or mildly increased ALT levels and to compare the predictive significance with that of other noninvasive fibrosis indicators, such as APRI and FIB-4. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5840/rc).

Highlight box

Key findings

- The constructed nomogram demonstrated outstanding diagnostic sensitivity, and it allows for a more visual and individualized assessment of the likelihood of SHCHI in chronic HBV-infected patients whose ALT levels are either normal or mildly increased.

What is known and what is new?

- Indicators for therapy in patients with chronic hepatitis B virus (HBV) infection include viremia and the severity of liver fibrosis and inflammation.
- This study aimed to create and verify a nomogram for detecting significant histopathologic changes in hepatic injury (SHCHI) in chronic HBV-infected patients exhibiting normal or mildly increased ALT levels and to compare the predictive significance with that of other noninvasive fibrosis indicators.

What is the implication, and what should change now?

- Both univariate and multivariate logistic regression analyses were utilized to conduct the risk factor screening for SHCHI. A nomogram was developed, and its accuracy was confirmed using the above two cohorts.

Methods

Patients

This was a retrospective study involving one center. From January 2017 to December 2021, data on consecutive patients with chronic HBV infection who did not receive antiviral treatment and had undergone liver biopsy at Yantai Qishan Hospital were collected. The following requirements were used for inclusion: HBV was identified by the presence of HBsAg for over 6 months; age >18 years; ALT ≤2 upper limit of normal (ULN; the ULN of ALT is 40 U/L); the results of standard laboratory tests and a liver biopsy; without antiviral treatment. The following criteria were used for exclusion: coinfection with human immunodeficiency virus (HIV), hepatitis D virus (HDV), or hepatitis C virus (HCV); accompanied with other chronic liver illnesses, including autoimmune liver disease or Wilson disease; with liver cirrhosis, decompensated liver disease, hepatocellular carcinoma, or other kinds of malignancies; or with incomplete clinical data. Finally, this research comprised 384 HBV-infected individuals who received liver biopsies. A total of 270 of these cases were classified into the training cohort for the construction of the nomogram, and the remaining 114 patients were classified into the validation cohort to determine how well the models performed. All 384 patients were randomly assigned to the two cohorts. The Institutional Committee of Yantai Qishan Hospital granted research approval (No. 202208) before it was conducted. Before participation in this study, informed consent was provided by each patient before their data was utilized. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Liver biopsy

The liver tissues were retrieved with the use of biopsy needles with a gauge size of 16 during a percutaneous liver biopsy that was guided by ultrasonography. Hematoxylin-eosin (HE) staining, as well as Gomori reticular fiber staining, were used on the samples after they were fixed and embedded in paraffin. The diagnostic procedure requires at least 1.5 cm of liver tissue with a minimum of 6 portal tracts. As per Scheuer's classification (15), two pathologists who were blinded to the clinical data performed the histological grading of necro-inflammation (G0–G4) and the staging of liver fibrosis (S0–S4). SHCHI were established as grade ≥ G2 inflammation and/or stage ≥ S2 fibrosis (16). According to the definition of SHCHI in literature 16, the five levels of inflammation and fibrosis were classified into two categories, but the five levels were not compared separately (*Figure 1*).

Clinical features

Within 3 days, comprehensive biochemical testing, ultrasonography, and liver biopsies were carried out on each patient. Before the biopsy of the liver was performed, blood samples were extracted. Patient data included age (at time of liver biopsy), gender, HBsAg (+) time (month), Family history, levels of ALT, AST, GGT, ALP, ALB, TBIL, PT, INR, FIB, WBC count, PLT count, number of HBeAg (+) cases, number of HBeAg (–) cases, logHBVDNA quantification, logHBsAg quantification, logHBeAg quantification, HBcAb quantification, and data on histopathological characteristics (*Table 1*). In our hospital's lab, biochemical testing was carried out using commercially available assays. A real-time polymerase chain reaction (RT-PCR) system (ABI7300, Applied Biosystems, Foster City, CA, USA) was utilized to ascertain the HBV DNA level in the serum with a lower limit of detection of 20 IU/mL. The level of 40 U/L was considered the upper limit of the control (ULC) range. An enzyme immunoassay based on microparticles was used in conjunction with a commercial kit (EIA, Abbott Laboratories, Chicago, IL, USA) to determine the HBeAg levels. There was a direct link between the level of HBeAg that was present in these samples and the relative light units (RLUs) that were determined through chemiluminescent reaction. The ratio of the RLU of the sample to that of the control cutoff (S/CO) was used to determine HBeAg levels. This led to the production of semiquantitative findings that were proportional to the HBeAg level. Titers of HBeAg with a value of >1.0 S/CO were considered to be positive, whereas titers of anti-HBeAg with a value of <1.0 S/CO were considered negative. After samples were diluted at 1:100, the Architect HBsAg assay (Abbott Laboratories; dynamic range 0.05–250 IU/mL) was used to determine the HBsAg level. When the HBsAg content of the above-mentioned sample (1:100 dilution) was more than 250 IU/mL, we diluted the sample with 1:1,000 and then detected it again.

Calculations for the non-invasive models were carried out using the following equations:

$$\text{APRI}=\left(\text{AST/ULN}\right)\times 100/\text{platelet count}\left(10^9/\text{L}\right) \quad [1]$$

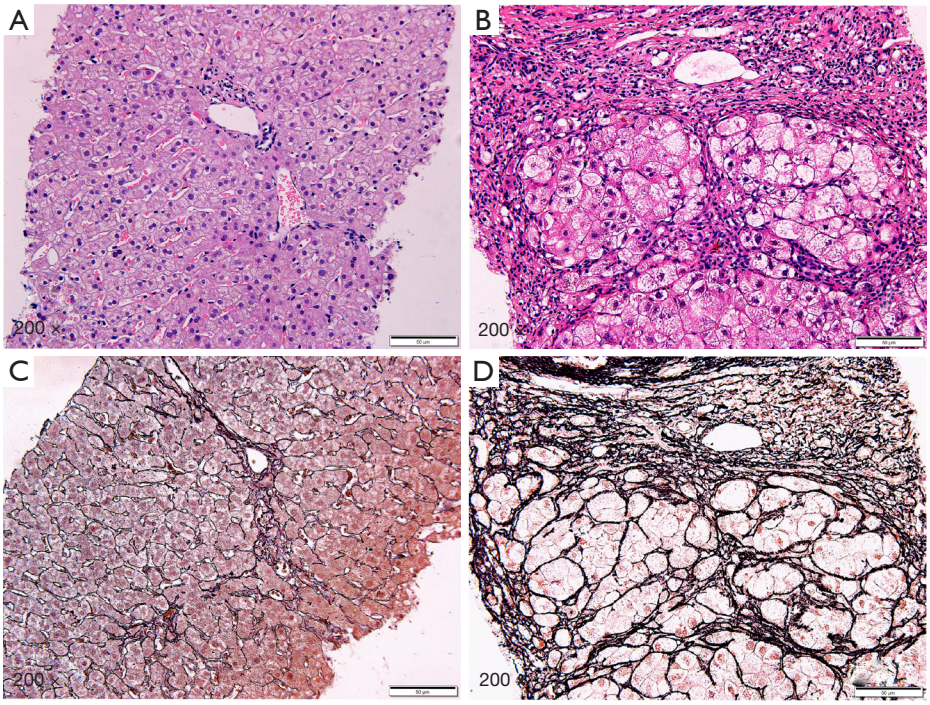

Figure 1 Hepatic pathological features in patients with hepatitis B viral infection (200×). (A) HE staining, inflammation grade < G2; (B) HE staining, inflammation grade ≥ G2; (C) Gomori reticular fiber staining, fibrotic stage < S2; (D) Gomori reticular fiber staining, fibrotic stage ≥ S2. HE, hematoxylin-eosin.

$$\text{FIB-4}=\left(\text{age(y)}\times\text{AST(U/L)}\right)/\left(\text{platelet count}\left(10^9/\text{L}\right)\times\left[\text{ALT(U/L)}^{[1/2]}\right]\right) \quad [2]$$

Statistical analysis

Our analysis illustrated that the continuous data [HBV-DNA, HBsAg, HBeAg, HBcAb, ALT, AST, GGT, ALP, total bilirubin (TBIL), prothrombin time (PT), international normalized ratio (INR)] were not normally distributed. Only the continuous data [age, HBsAg (+) time] conformed to normal distribution. Consequently, the normally distributed continuous data were presented as mean ± standard deviation ($\bar{x}$ ± SD), and the others were presented as median [interquartile range (IQR)]. The differences (variations) across groups were subjected to a comparison by *t*-test or Mann-Whitney U test. The categorical data were described by frequency, and variations across groups were contrasted by the χ^2 test or Fisher's exact test.

A priori analysis was undertaken to determine the risk variables linked to SHCHI. This analysis was based on the clinical significance, scientific comprehension, and predictors discovered in previous research papers (17,18). Univariate and multivariate logistic regression analyses were conducted to analyze the risk factors. All variables were grouped via three methods before univariate logistic analysis. First, the areas under the receiver operating characteristic (ROC) curve were employed to determine the diagnostic value of indexes for SHIHC, including age, ALT, AST, GGT, ALP, ALB, TBIL, PT, INR, and HBcAb. Second, optimal scale regression was utilized to examine the diagnostic value of HBeAg and HBsAg. Third, HBV DNA was grouped according to published literature, gender was grouped by male/female, and family history was divided by yes or no. In the multivariate analysis of logistic regression, we only considered those variables that had shown statistical significance in the univariate analysis (P<0.05), and the forward stepwise technique, also known as the likelihood ratio test, was utilized in selecting the variables that would ultimately be incorporated into the model. Tolerance (TOL) or variance inflation factor (VIF) was used to evaluate plausible interaction terms before multivariate logistic regression analysis. As there was no evidence of significant interactions, the multivariable analysis did not include any interaction terms.

By employing the *rms* package included in R, version

Table 1 Baseline characteristics of enrolled patients

Variables	Training cohort (N=270)	Validation cohort (N=114)	P value
Gender, n (%)			
Male	159 (58.89)	79 (69.30)	0.055
Female	111 (41.11)	35 (30.70)	
Age (years), mean ± SD	45.71±10.93	44.31±10.44	0.246
HBsAg (+) time (month), mean ± SD	96.20±97.24	105.73±101.55	0.132
Family history (yes), n (%)	84 (31.11)	36 (31.58)	0.928
ALT (U/L), median (IQR)	38.00 (29.03)	40 (25.33)	0.009
AST (U/L), median (IQR)	29.00 (18.00)	39.2 (17.47)	0.578
GGT (U/L), median (IQR)	24.65 (32.25)	43.56 (24.95)	0.028
ALP (U/L), median (IQR)	75.25 (26.00)	76.62 (20.03)	0.417
ALB (g/L), median (IQR)	43.00 (6.00)	43.52 (5.46)	0.063
TBIL (μmol/L), median (IQR)	17.05 (10.34)	16.25 (11.19)	0.302
PT (s), median (IQR)	12.00 (1.80)	12.6 (1.2)	0.000
INR, median (IQR)	0.97 (0.12)	1.02 (0.1)	0.003
FIB (g/L), mean ± SD	2.83±0.47	2.71±0.53	0.023
WBC (10^9/L), mean ± SD	5.35±1.71	6.38±2.38	0.000
PLT (10^9/L), mean ± SD	174.72±71.08	167.21±55.42	0.315
HBeAg (+), n (%)	143 (52.96)	68 (59.65)	0.229
HBeAg (–), n (%)	127 (47.04)	46 (40.35)	
logHBVDNA (IU/mL), median (IQR)	3.63 (3.76)	4.37 (4.54)	0.796
logHBsAg (IU/mL), median (IQR)	3.56 (1.06)	3.55 (0.95)	0.256
logHBeAg (S/CO), median (IQR)	0.16 (3.31)	0.67 (2.46)	0.642
HBcAb (S/CO), mean ± SD	8.98±2.02	8.91 (2.24)	0.455
Grading of inflammation, n (%)			
G0	3 (1.1)	1 (0.89)	1.000
G1	82 (30.4)	28 (24.56)	0.250
G2	112 (41.5)	59 (51.75)	0.064
G3	67 (24.8)	24 (21.05)	0.428
G4	6 (2.2)	2 (1.75)	1.000
Stage of liver fibrosis, n (%)			
S0	9 (3.3)	11 (9.65)	0.011
S1	90 (33.3)	41 (35.97)	0.619
S2	72 (26.7)	40 (35.09)	0.097
S3	60 (22.2)	13 (11.40)	0.000
S4	39 (14.4)	9 (7.89)	0.076
SHCHI ≥ G2 and/or ≥ S2, n (%)	201 (74.44)	87 (76.32)	0.699

SD, standard deviation; HBsAg, hepatitis B surface antigen; ALT, alanine transaminase; IQR, interquartile range; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; ALB, albumin; TBIL, total bilirubin; PT, prothrombin time; INR, international normalized ratio; FIB, fibrinogen; WBC, white blood cell; PLT, platelet; HBeAg, hepatitis B e antigen; HBVDNA, Hepatitis B virus deoxyribonucleic acid; SHCHI, significant histopathologic changes of hepatic injury.

4.1.0 (http://www.r-project.org/; the R Foundation for Statistical Computing, Vienna, Austria) (17), a nomogram was created premised on the findings of a multivariate analysis. The nomogram was derived from the process of proportionally transforming each regression coefficient. The predictive ability of the nomogram was verified utilizing the concordance index (C-index), and its accuracy was further calibrated using 40 bootstrap samples to reduce the degree of overfitting bias.

By employing the StataSE-64 software, we compared the AUC values between different diagnostic models (nomogram APRI and FIB-4). The accuracy of APRI and FIB-4 for the diagnosis of SHCHI were evaluated depending on the values for the area under the ROC curves (AUC) and maximizing Youden's index led to the identification of the optimum cut-off points. The evaluation of the diagnostic performance was based on the specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The predictive values, specificity, sensitivity, and likelihood ratios were utilized to determine the accuracy of the optimal cutoff value.

The software SPSS 22.0 (IBM Corp., Armonk, NY, USA) and R version 4.1.0 were utilized to execute all analyses of statistical data. Statistical significance was set at two-side P<0.05.

Results

Baseline features of patients included in the study

During this study period, ultrasound percutaneous liver biopsy was performed on 472 patients (training cohort, n=341; validation cohort n=131) who had chronic HBV infection. Some 384 patients who were eligible to participate based on the set of criteria for inclusion were involved and categorized into the training (n=270) and validation (n=114) cohorts.

Table 1 outlines the various characteristics of the patients who belonged to the training and validation cohorts. The SHCHI were found in 201 (74.44%) and 87 (76.32%) patients in the two cohorts (P=0.699), respectively (*Table 1*).

Logistic regression analysis of SHCHI in the training cohort

As per Scheuer's classification, the 270 patients in the training cohort were split into two groups: a group with significant histopathologic changes of hepatic injury (n=69 instances) and a group with no such changes (n=201). The results of univariate logistic analysis illustrated remarkable differences in age, HBeAg, HBcAb, ALT, AST, GGT, ALP, TBIL, HBV DNA, PT INR, and FIB between the two groups (all P<0.05, *Table 2*). The multivariate analysis regression was to include these variables. Since 1 of the HBsAg positive time (months) groups (<168 *vs.* >252, P=0.866) and 1 of the HBsAg, IU/mL groups (<8,000 *vs.* 8,000–38,000, P=0.60) did not exhibit any significant differences (P>0.05), the 2 variables were excluded (*Table 2*). Based on the findings of multivariate analysis, it was shown that HBeAg, AST, PT, and GGT, were independently associated with SHCHI (all P<0.05, *Table 3*).

Establishment and verification of an SHCHI-diagnosing nomogram

By taking SHCHI as the dependent variable and HBeAg, AST, PT, and GGT as the independent variables, a nomogram was developed with the help of the R software (*Figure 2A*). *Figure 2B* shows that the nomogram had a C-index of 0.80, indicating that it had a strong capacity for discriminating and excellent accuracy in evaluating the risk of SHCHI.

The bootstrap approach was selected for use in the training cohort as the procedure for conducting internal verification. There were a total of 40 repetitions of the sampling procedure, and the error probability was calculated to be 0.011 on average. The calibration line within the calibration plot visually demonstrated that there was a strong consistency in the existence of SHCHI between the risk assessment provided by the nomogram and the histopathologic diagnosis obtained from the liver biopsy. When applied to the validation cohort, the nomogram showed a C-index of 0.75 when calculating the SHCHI risk (*Figure 2C*). Furthermore, there was a reasonable calibration curve for the risk estimation as depicted in *Figure 2C*. Collectively, a high level of accuracy was achieved by the nomogram developed for the diagnosis of SHCHI.

Diagnostic Values of the Nomogram Compared with ARPI and FIB-4

In terms of the AUC values for predicting significant histological changes, the nomogram outperformed APRI (0.80 *vs.* 0.78, P=0.527, and FIB-4 (0.80 *vs.* 0.76, P=0.272) (*Table 4* and *Figure 3*). The optional cut-off values for

Table 2 Univariate logistic regression analysis of SHCHI in the training cohort

Variables	β	Wald	OR	95% CI	P value
Gender (male *vs.* female)	−0.110	0.150	0.895	0.512–1.566	0.698
Age (years) (≤49 *vs.* >49)	0.888	7.447	2.430	1.284–4.598	0.006
Family history (yes *vs.* no)	0.425	1.797	1.529	0.822–2.846	0.180
HBsAg positive time (months)					
<168 *vs.* 169–252	−0.752	4.617	0.472	0.238–0.936	0.032
<168 *vs.* >252	−0.116	0.028	0.891	0.232–3.419	0.866
HBeAg (positive *vs.* negative)	−0.036	0.16	0.965	0.558–1.670	0.899
HBsAg (IU/mL)					
<8,000 *vs.* 8,000–38,000	−0.700	3.529	0.497	0.239–1.031	0.60
<8,000 *vs.* >38,000	−1.851	21.910	0.157	0.072–0.341	0.000
HBeAg (S/CO)					
<700 *vs.* 700–1,600	−1.336	6.914	0.263	0.097–0.712	0.009
<700 *vs.* >1,600	−3.071	27.666	0.046	0.015–0.146	0.000
HBcAb (S/CO) (≤7.935 *vs.* >7.935)	0.801	7.177	2.229	1.240–4.005	0.007
ALT (U/L) (≤21.08 *vs.* >21.08)	0.868	7.322	2.383	1.270–4.470	0.007
AST (U/L) (≤29.15 *vs.* >29.15)	1.312	18.031	3.713	2.026–6.802	0.000
GGT (U/L) (≤30.46 *vs.* >30.46)	1.299	15.396	3.664	1.915–7.010	0.000
ALP (U/L) (≤108 *vs.* >108)	1.534	6.146	4.639	1.379–15.603	0.013
TBIL (μmol/L) (≤14.1 *vs.* >14.1)	0.709	6.132	2.032	1.159–3.563	0.013
PT (s) (≤12.65 *vs.* >12.65)	1.545	16.102	4.689	2.204–9.975	0.000
INR (≤0.98 *vs.* >0.98)	1.088	12.088	2.969	1.608–5.483	0.001
HBVDNA (U/mL)					
<20 *vs.* 20–2,000	−1.440	4.866	0.237	0.066–0.852	0.027
<20 *vs.* ≥2,000	−1.733	7.678	0.177	0.052–0.602	0.006

SHCHI, significant histopathologic changes of hepatic injury; β, regression coefficient; Wald, Wald test; OR, odds ratio; 95% CI, confidence interval; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; S/CO, the ratio of the RLU (the relative light units) of the sample to that of the control cutoff; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; TBIL, total bilirubin; PT, prothrombin time; INR, international normalized ratio; HBVDNA, hepatitis B virus deoxyribonucleic acid.

Table 3 Multivariate logistic regression analysis of SHCHI in the training cohort

Variables	β	Wald	P value	OR	95% CI
HBeAg (S/CO)					
≤700 *vs.* 700–1,600	−0.831	4.753	0.029	0.435	0.206–0.920
≤700 *vs.* >1,600	−2.360	9.667	0.002	0.094	0.021–0.418
AST (U/L) (≤29.15 *vs.* >29.15)	1.028	8.398	0.004	2.794	1.395–5.599
PT (s) (≤12.65 *vs.* >12.65)	1.694	14.928	0.000	5.443	2.304–12.855
GGT (U/L) (≤29.15 *vs.* >29.15)	1.048	7.789	0.005	2.853	1.366–5.958

SHCHI, significant histopathologic changes of hepatic injury; β, regression coefficient; Wald, Wald test; OR, odds ratio; CI, confidence interval; HBeAg, hepatitis B e antigen; S/CO, The ratio of the RLU (the relative light units) of the sample to that of the control cutoff; AST, aspartate aminotransferase; PT, prothrombin time; GGT, γ-glutamyl transferase.

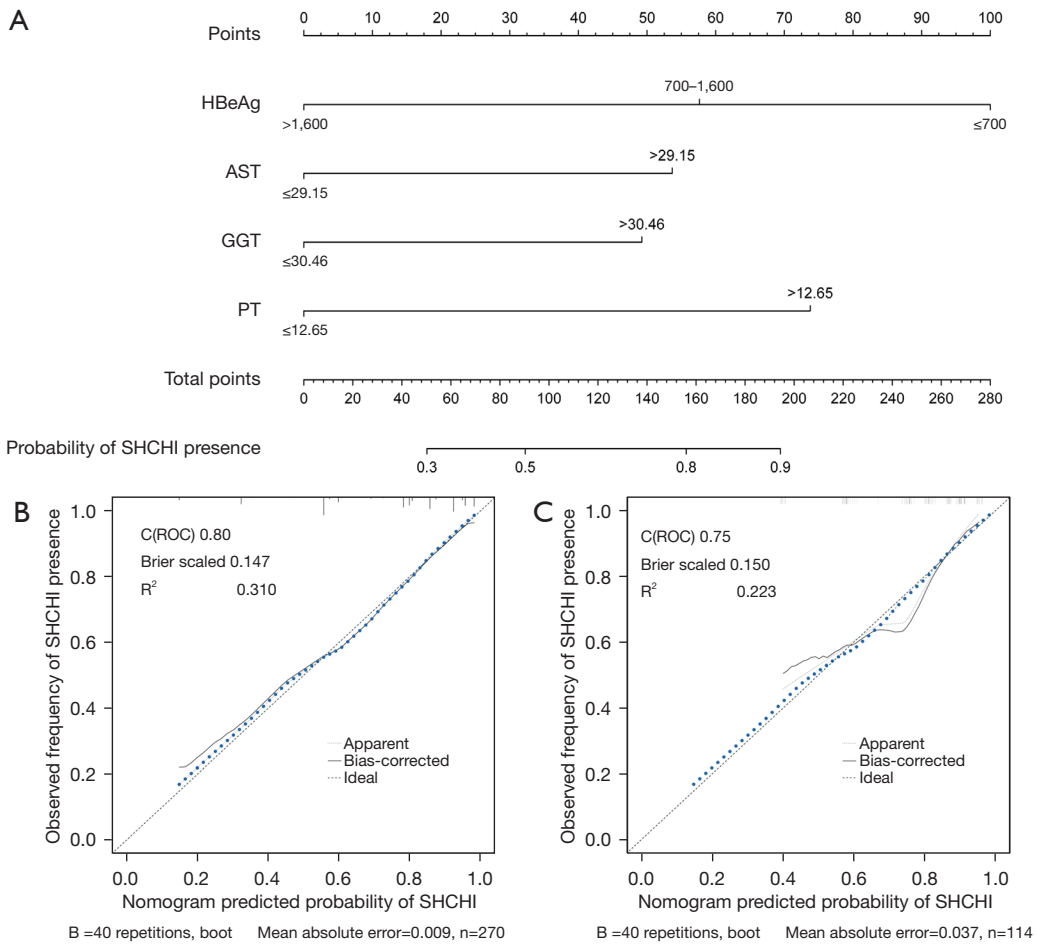

Figure 2 Construction and verification of a SHCHI-diagnosing nomogram. (A) Nomogram to measure the risk of SHCHI in patients with chronic HBV infection exhibiting alanine transaminase levels that are normal or mildly increased. To make use of the nomogram, locate the value of each variable along the axis that corresponds to it, create a line to the axis of points to represent the number of points, compile the values from each of the different variables, and create a line starting from the axis that represents the total number of points to figure out the SHCHI risks at the bottom line of the nomogram. A greater total score suggested a higher likelihood of SHCHI diagnosis. (B) Calibration plot illustrating the predictive ability of the nomogram in determining the likelihood of SHCHI being present in the training cohort (n=270). Illustration of SHCHI prediction based on the 40-sample bootstrapped calibration plot. C(ROC) is used to calculate the C index to examine the discrimination of the nomogram. The Brier scale evaluates the calibration degree of the nomogram, and R^2 is the fitting degree of the nomogram. The ideal line represents the ideal fit; the bias-corrected line signifies the bootstrap-corrected estimate values; the apparent line highlights nomogram-predicted probabilities. (C) Validity of the prediction accuracy of the nomogram in assessing the risk of SHCHI presence in the validation cohort (n=114). HBeAg, hepatitis B e antigen; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; PT, prothrombin time; SHCHI, significant histopathologic changes of hepatic injury; C(ROC), C index, concordance index; ROC, receiver operating characteristic.

predicting substantial histological changes were obtained by determining the results of maximizing Youden's index. These values were 119 for the nomogram, 0.38 for the APRI, and 1.18 for the FIB-4. The accuracy of the nomogram, APRI, and FIB-4 in diagnosing SHCHI were 76.29%, 73.67%, and 70.78%, respectively (*Table 4* and *Figure 3*). The sensitivity, specificity, PPV, and NPV were 79.10%, 68.10%, 87.84%, and 52.80%, respectively, when the nomogram was utilized to distinguish the presence and absence of SHCHI.

Discussion

HBV infections pose a significant burden on both

Table 4 The diagnostic performance of markers for diagnosing SHCHI

Models	AUC	95% CI	Cut-off	Se (%)	Sp (%)	PLR	NLR	PPV (%)	NPV (%)	Accuracy (%)
Nomogram	0.80	0.74–0.86	119	79.10	68.10	2.48	0.31	87.84	52.80	76.29
APRI*	0.78	0.71–0.84	0.38	72.60	76.80	3.13	0.36	90.11	49.04	73.67
FIB-4*	0.76	0.69–0.82	1.18	68.20	78.30	3.14	0.41	90.15	45.81	70.78

*, AUROC of APRI and FIB-4 were no significant difference in comparison with Nomogram, P>0.05. SHCHI, significant histopathologic changes of hepatic injury; APRI, aspartate transaminase to platelet ratio index, FIB-4, fibrosis index based on the four factors; AUC, area under the receiver operating characteristic curve; CI, confidence interval; Se, sensitivity; Sp, specificity; PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

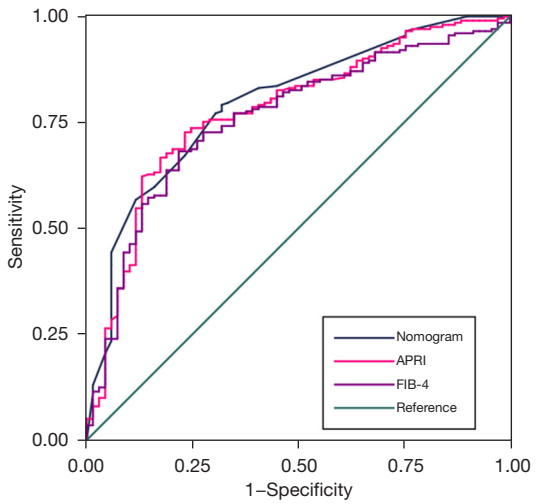

Figure 3 ROC plot for nomogram, FIB-4, and APRI in diagnosing SHCHI patients in the training cohort (n=270). AUROCs of Nomogram, FIB-4 and APRI were 0.80, 0.76, 0.78, respectively. ROC, receiver operating characteristic; FIB-4, Fibrosis-4 scoring model; APRI, the AST-to-PLT ratio index scoring model; SHCHI, significant histopathologic changes of hepatic injury; AUROCs, the areas under the ROC curves; AST, aspartate aminotransferase; PLT, platelet.

public health and the economy across the world, with approximately 257 million individuals worldwide experiencing chronic infection (19-21). Depending on the natural progression course of the illness, chronic HBV infection and chronic hepatitis B (CHB) are often broken down into 4 distinct phases throughout their respective natural histories: the immune-tolerant period (chronic HBV carrier state), immune clearance period (HBeAg-positive CHB), immune control period (inactive HBsAg carrier state), and reactivation period (HBeAg-negative CHB) (21). The immune-tolerant phase is the most fascinating and, according to our expectations, the least understood (3) chronic HBV carrier, which as a state is also in dynamic change. Over time, the immune tolerance state will spontaneously be completely or not completely broken and be taken to the immune clearance stage, with liver tissue immune injury, so that the disease gradually progresses, or even advances to cirrhosis or hepatocellular carcinoma (20). Does the patient with positive HBV DNA and normal or mildly elevated ALT have to be in an immune tolerance period? A few recent studies demonstrated that people with "normal" ALT levels can experience liver histological abnormalities and may necessitate antiviral treatment (6). In a study from the Netherlands (7) that included 2,991 patients, significant liver fibrosis occurred in 7.2% of patients having ALT <1 ULN and in 25% of patients whose ALT was 1–2 ULN. In addition, a retrospective study of 253 patients with CHB who received liver biopsies from 1990 to 2009 at the DÜSSELDORF in Germany (22) showed that among untreated patients with ALT <1 ULN or 1–2 ULN, the prevalence of fibrosis and cirrhosis was 35.9% and 17.9%. As per these findings, in a European context, patients with CHB infection who have normal transaminase levels typically have substantial liver cirrhosis or fibrosis (22). Back in 2010, research involving 522 patients with newly diagnosed CHB who received liver biopsy showed that a high proportion (21–30 years old, 17.4%) of young patients (≤30 years old) with normal ALT still had significant histopathological changes (5). It might be that these patients still need to be tested for histologic liver disease and treated accordingly to slow disease progression and achieve long-term clinical benefits. Notably, some patients with HBeAg-negative could not express HBeAg because of gene mutation, but still had significant liver inflammation and replicating fibrosis (23-25). A study of HBeAg-negative patients showed that 43 patients (35.9%) had a fibrotic stage ≥2 whereas 48 patients (40%) had a fibrotic stage ≥2 and/or hepatic

activity index (HAI) ≥6 (24). Furthermore, the results of another review (25) illustrated that among 9 studies (n=830 patients), a high percentage [20.7%; 95% confidence interval (CI): 16.2–26.0%] of CHB patients who had ALT levels ≤40 U/L exhibited severe fibrosis regardless of their HBeAg status. Currently, with the lack of novel indicators that can distinguish between the distinct stages of the natural history of chronic HBV infection, liver histology continues to be the golden criterion for measuring whether or not a patient with normal or mildly elevated ALT levels needs therapy. However, due to the invasive nature, liver biopsies are generally not performed in clinical practice if the patient's ALT level is normal or mildly elevated. Moreover, it is not enough to monitor ALT levels for these patients, so they are often ignored (26,27). Recently, although there have been various indicators that have been reported to be the alternative for a biopsy, the accuracy of the evaluation of liver fibrosis and liver inflammation has been contentious. As such, this paper aimed to create a simple and convenient nomogram for diagnosing SHCHI in patients with chronic HBV infection exhibiting normal or mild levels of ALT and to verify its diagnostic performance.

Based on the findings of the univariate and multivariate logistic regression analyses, after adjusting factors for age, HBsAg positive time (months), HBcAb, ALT, ALP, INR, and HBV-DNA, HBeAg, AST, GGT, and PT were found to independently serve as risk indicators for SHCHI [odds ratio (OR) and 95% CI of 0.965 (0.558–1.670), 3.713 (2.026–6.802), 3.664 (1.915–7.010), 4.689 (2.204–9.975)]. Patients with persistent HBV infection who had ALT levels that were either normal or mildly elevated had a good concordance between the SHCHI-nomogram and liver biopsies that predicted histological alterations (21).

Compared with the other nomograms, our study has several strengths such as a wider application range and greater suitability for clinical practice. Chang *et al.* (28) and Wang *et al.* (29) established a nomogram or non-invasive model of chronic HBV infection with normal ALT which aimed at the HBeAg-positive patients but did not include HBeAg-negative cases. Therefore, the application of the models was limited to a certain extent. In our study, the occurrence of SHCHI was independent of HBeAg positivity or negativity, and HBeAg was quantitatively modeled as a whole regardless of positive or negative status, which allows the model to be used more widely. In addition, the patients selected in this paper were not excluded due to steatosis, which was more practical and in line with the actual situation of clinical patients. The diagnostic performance of the newly created nomogram was not hindered by the presence of steatosis, in contrast with the FibroScan, which was hampered by this constraint. Further, the 4 variables included in our model were all routinely available clinical parameters.

In the past decade, numerous non-invasive models for diagnosing HBV-infection-related liver fibrosis have emerged. Nonetheless, the majority of non-invasive diagnostic methods were derived from chronic hepatitis C, and their accuracy is limited to identifying cirrhosis from diseases characterized by little or mild fibrosis (30-32). When applied to estimate the extent of liver fibrosis in individuals with CHB, such models have produced conflicting findings (33). In a systematic review, both FIB-4 and APRI were shown to have a reasonable level of sensitivity and accuracy when it came to diagnosing fibrosis caused by HBV in a systematic review (34). The AUCs for patients with normal ALT were 0.71 and 0.72, respectively (34). Compared with the diagnostic efficiency of APRI and FIB-4, the diagnostic efficiency of the nomogram (AUC 0.80) was greater compared to the AUC of APRI and FIB-4 (0.78, 0.76). Although the differences were not statistically significant (nomogram *vs.* APRI, P=0.527; nomogram *vs.* FIB-4, P=0.272), the nomogram was more accurate in diagnosing SHCHI than APRI and FIB-4.

When assessing clinical applications of the model in measuring the risk of SHCHI, we analyzed the specificity, sensitivity, NPV, and PPV, with 119 serving as the cutoff point. Patients with a score of 119 or above were considered members of the SHCHI high-risk group (PPV, 87.84%). The nomogram, which is premised on predictions, could be used as a resource to additionally examine the state of immunological tolerance-period patients and HBeAg-negative patients with normal ALT, as well as to identify which individuals should receive antiviral medication.

It is important to emphasize that the dependability of the nomogram will necessitate further confirmation in a prospective study for our research to be considered conclusive. In addition, this investigation relied on the data from a single center; hence, it is essential to confirm the findings from multiple centers. Furthermore, although the nomogram had an excellent prediction accuracy with a cutoff value of 119, it had a false-positive rate of 31.9% and a false-negative rate of 20.9%, therefore, comprehensive consideration is still needed before making clinical decisions.

Conclusions

A nomogram was created by incorporating 4 SHCHI-

related primary risk variables. The model gives an appropriate assessment of the risk of SHCHI in patients who have chronic HBV infection and ALT levels that are either normal or mildly increased. In addition, the nomogram performed well on internal validation and was superior to some other non-invasive models (including APRI, and FIB-4). For people in the immune tolerance stage or with normal or mildly elevated ALT, guidelines recommend that treatment depends on liver biopsy results and a comprehensive evaluation (21). Our nomogram could provide an accurate, practical, convenient, reliable, and non-invasive evaluation tool for this population. Moreover, it could also replace liver biopsy to dynamically assess the progress of the disease in this population. This would offer a novel chance for targeted, early HBV therapy in patients for whom it is urgently required, with the ultimate goal of reducing complications linked to HBV. To prove the nomogram's utility in identifying SHCHI risk in patients with chronic HBV infection and ALT levels that are normal or only moderately raised, additional studies will need to be conducted in the future to perform an external validation of the nomogram.

Acknowledgments

We thank the patients at the Yantai Qishan Hospital .
Funding: None.

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5840/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5840/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5840/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics board of Yantai Qishan Hospital (No. 202208). The informed consent was obtained from the patients before participation in this study.

References

1. Yim HJ, Kim JH, Park JY, et al. Comparison of clinical practice guidelines for the management of chronic hepatitis B: When to start, when to change, and when to stop. Clin Mol Hepatol 2020;26:411-29.
2. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560-99.
3. Wong GL. Management of chronic hepatitis B patients in immunetolerant phase: what latest guidelines recommend. Clin Mol Hepatol 2018;24:108-13.
4. Choi GH, Kim GA, Choi J, et al. High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation. Aliment Pharmacol Ther 2019;50:215-26.
5. Gui HL, Wang H, Yang YH, et al. Significant histopathology in Chinese chronic hepatitis B patients with persistently high-normal alanine aminotransferase. J Viral Hepat 2010;17 Suppl 1:44-50.
6. Nguyen MH, Garcia RT, Trinh HN, et al. Histological disease in Asian-Americans with chronic hepatitis B, high hepatitis B virus DNA, and normal alanine aminotransferase levels. Am J Gastroenterol 2009;104:2206-13.
7. Sonneveld MJ, Brouwer WP, Hansen BE, et al. Very low probability of significant liver inflammation in chronic hepatitis B patients with low ALT levels in the absence of liver fibrosis. Aliment Pharmacol Ther 2020;52:1399-406.
8. Varela-Ponte R, Martínez-Lago N, Vieito-Villar M, et al. Impact of risk factors on the efficacy and complications of ultrasound-guided percutaneous liver biopsy of space-

occupying lesions. Radiologia (Engl Ed) 2022;64:497-505.
9. Midia M, Odedra D, Shuster A, et al. Predictors of bleeding complications following percutaneous image-guided liver biopsy: a scoping review. Diagn Interv Radiol 2019;25:71-80.
10. Chen Y, Gong J, Zhou W, et al. A Novel Prediction Model for Significant Liver Fibrosis in Patients with Chronic Hepatitis B. Biomed Res Int 2020;2020:6839137.
11. Teshale E, Lu M, Rupp LB, et al. APRI and FIB-4 are good predictors of the stage of liver fibrosis in chronic hepatitis B: the Chronic Hepatitis Cohort Study (CHeCS). J Viral Hepat 2014;21:917-20.
12. Wu J, Guo N, Zhang X, et al. HEV-LF(S) : A novel scoring model for patients with hepatitis E virus-related liver failure. J Viral Hepat 2019;26:1334-43.
13. Chen L, Zhang J, Lu T, et al. A nomogram to predict survival in patients with acute-on-chronic hepatitis B liver failure after liver transplantation. Ann Transl Med 2021;9:555.
14. Li S, Zhang X, Li Q, et al. Development and validation of the nomogram based on INR and eGFR for estimation of mortality in patients with acute-on-chronic hepatitis B liver failure. BMC Gastroenterol 2021;21:474.
15. Scheuer P. Primary biliary cirrhosis. Proc R Soc Med 1967;60:1257-60.
16. Consensus on the diagnosis and treatment of hepatic fibrosis (2019). J Dig Dis 2020;21:127-38.
17. Zhang ZQ, Wang GS, Kang KF, et al. Nomogram for hepatic steatosis: A simple and economical diagnostic tool for massive screening. Dig Liver Dis 2016;48:914-20.
18. Cheng D, Wan G, Sun L, et al. A Novel Diagnostic Nomogram for Noninvasive Evaluating Liver Fibrosis in Patients with Chronic Hepatitis B Virus Infection. Biomed Res Int 2020;2020:5218930.
19. Mysore KR, Leung DH. Hepatitis B and C. Clin Liver Dis 2018;22:703-22.
20. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384:2053-63.
21. Chinese Society of Infectious Diseases, Chinese Medical Association; Chinese Society of Hepatology, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). Zhonghua Gan Zang Bing Za Zhi 2019;27:938-61.
22. Göbel T, Erhardt A, Herwig M, et al. High prevalence of significant liver fibrosis and cirrhosis in chronic hepatitis B patients with normal ALT in central Europe. J Med Virol 2011;83:968-73.
23. Papatheodoridis GV, Manolakopoulos S, Liaw YF, et al. Follow-up and indications for liver biopsy in HBeAg-negative chronic hepatitis B virus infection with persistently normal ALT: a systematic review. J Hepatol 2012;57:196-202.
24. Ormeci A, Aydın Y, Sumnu A, et al. Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels. Int J Infect Dis 2016;52:68-73.
25. Chao DT, Lim JK, Ayoub WS, et al. Systematic review with meta-analysis: the proportion of chronic hepatitis B patients with normal alanine transaminase ≤ 40 IU/L and significant hepatic fibrosis. Aliment Pharmacol Ther 2014;39:349-58.
26. Gong X, Yang J, Tang J, et al. A Mechanistic Assessment of the Discordance between Normal Serum Alanine Aminotransferase Levels and Altered Liver Histology in Chronic Hepatitis B. PLoS One 2015;10:e0134532.
27. Wang H, Ru GQ, Yan R, et al. Histologic Disease in Chinese Chronic Hepatitis B Patients With Low Viral Loads and Persistently Normal Alanine Aminotransferase Levels. J Clin Gastroenterol 2016;50:790-6.
28. Chang X, Wang J, Chen Y, et al. A novel nomogram to predict evident histological liver injury in patients with HBeAg-positive chronic hepatitis B virus infection. EBioMedicine 2021;67:103389.
29. Wang H, Zhou Y, Yan R, et al. Development and validation of a model for staging hepatic fibrosis for chronic hepatitis B patients with E antigen-positive. Oncotarget 2017;8:98812-22.
30. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.
31. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26.
32. Lesmana CR, Salim S, Hasan I, et al. Diagnostic accuracy of transient elastography (FibroScan) versus the aspartate transaminase to platelet ratio index in assessing liver fibrosis in chronic hepatitis B: the role in primary care setting. J Clin Pathol 2011;64:916-20.
33. Wang L, Li J, Yang K, et al. Comparison and evaluation of non-invasive models in predicting liver inflammation and fibrosis of chronic hepatitis B virus-infected patients with high hepatitis B virus DNA and normal or mildly

elevated alanine transaminase levels. Medicine (Baltimore) 2020;99:e20548.
34. Wang H, Xue L, Yan R, et al. Comparison of FIB-4 and APRI in Chinese HBV-infected patients with persistently normal ALT and mildly elevated ALT. J Viral Hepat 2013;20:e3-10.

(English Language Editor: J. Jones)

Cite this article as: Sun LL, Wang M, Zhang NN, Chen MX, Li YY, Zhang S, Qin CY. Establishment and validation of a diagnostic nomogram for significant histopathologic changes of hepatic injury in HBV-infected patients. Ann Transl Med 2023;11(2):40. doi: 10.21037/atm-22-5840