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A non-invasive model for predicting liver fibrosis in HBeAg-positive patients with normal or slightly elevated alanine aminotransferase

Ling Li, PhD[a,b], Yongan Ye, PhD[a], Yun Ran, PhD[b], Shuyan Liu, PhD[c], Qiyuan Tang, MD[c], Yaya Liu, MD[c], Xuejiao Liao, MD[c], Juanjuan Zhang, MD[c], Guohui Xiao, PhD[c], Jian Lu, PhD[d], Guoliang Zhang, PhD[c], Qing He, PhD[c], Shiping Hu, PhD[b,*]

Abstract

Early and accurate diagnosis of liver fibrosis is necessary for HBeAg-positive chronic hepatitis B (CHB) patients with normal or slightly increased alanine aminotransferase (ALT), Liver biopsy and many non-invasive predicting markers have several application restrictions in grass-roots hospitals. We aimed to construct a non-invasive model based on routinely serum markers to predict liver fibrosis for this population.

A total of 363 CHB patients with HBeAg-positive, ALT ≤2-fold the upper limit of normal and liver biopsy data were randomly divided into training (n = 266) and validation groups (n = 97). Two non-invasive models were established based on multivariable logistic regression analysis in the training group. Model 2 with a lower Akaike information criterion (AIC) was selected as a better predictive model. Receiver operating characteristic (ROC) was used to evaluate the model and was then independently validated in the validation group.

The formula of Model 2 was logit (Model value) = 5.67+0.08 × Age −2.44 × log10 [the quantification of serum HBsAg (qHBsAg)] −0.60 × log10 [the quantification of serum HBeAg (qHBeAg)]+0.02 × ALT+0.03 × aspartate aminotransferase (AST). The area under the ROC curve (AUC) was 0.89 for the training group and 0.86 for the validation group. Using 2 cut-off points of −2.61 and 0.25, 59% of patients could be identified with liver fibrosis and antiviral treatment decisions were made without liver biopsies, and 149 patients were recommended to undergo liver biopsy for accurate diagnosis.

In this study, the non-invasive model could predict liver fibrosis and may reduce the need for liver biopsy in HBeAg-positive CHB patients with normal or slightly increased ALT.

Abbreviations: AIC = Akaike information criterion, ALT = alanine aminotransferase, AST = aspartate aminotransferase, AUC = the area under the ROC curve, CHB = chronic hepatitis B, HBeAg = hepatitis B e antigen, HBsAg = hepatitis B surface antigen, HBV DNA = hepatitis B virus deoxyribonucleic acid, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HDV = hepatitis D virus, HIV = human immunodeficiency virus, NPV = negative predictive value, PPV = positive predictive value, qHBeAg = the quantification of serum HBeAg, qHBsAg = the quantification of serum HBsAg, ROC = receiver operating characteristic curve, ULN = the upper limit of normal.

Keywords: chronic hepatitis B, HBeAg-positive, liver fibrosis, non-invasive predicting model, sensitivity and specificity

Editor: Dongbo Wu.

LL, YY, and YR contributed equally to this work.

This study was supported by the National Natural Science Foundation of China (Item number: 81873958 and 81973733), Natural Science Foundation of Guangdong Province (Item number: 2019A1515011555), Shenzhen Scientific and Technological Foundation (Item number: JCYJ20170412151620658, JCYJ20170307095003051, and JCYJ20170306154458909), and the Sanming Project of Medicine in Shenzhen (Item number: SZSM201911009).

The datasets used and analyzed in the current study are available from the corresponding author upon request.

This study was approved by the Medical Research Ethics Committee at The Third People's Hospital of Shenzhen in accordance with the principles laid down in the Declaration of Helsinki (1975).

Supplemental Digital Content is available for this article.

The authors have no conflicts of interests to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

[a] Department of Gastroenterology and Hepatology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, [b] Department of Hepatology, Beijing University of Chinese Medicine Affiliated Shenzhen Hospital, [c] National Clinical Research Center for Infectious Diseases, Shenzhen Third People's Hospital, Southern University of Science and Technology, [d] Department of Infectious Diseases, Shenzhen University General Hospital, Shenzhen, China.

[*] Correspondence: Shiping Hu, Department of Hepatology, Beijing University of Chinese Medicine Affiliated Shenzhen Hospital, Beijing University of Chinese Medicine, Shenzhen 518172, Guangdong, China (e-mail: 20170941077@bucm.edu.cn).

How to cite this article: Li L, Ye Y, Ran Y, Liu S, Tang Q, Liu Y, Liao X, Zhang J, Xiao G, Lu J, Zhang G, He Q, Hu S. A non-invasive model for predicting liver fibrosis in HBeAg-positive patients with normal or slightly elevated alanine aminotransferase. Medicine 2021;100:17(e25581).

Received: 9 October 2020 / Received in final form: 24 February 2021 / Accepted: 30 March 2021

http://dx.doi.org/10.1097/MD.0000000000025581

1. Introduction

Chronic hepatitis B virus (CHB) infection is usually a precursor to progressive chronic liver inflammation, hepatic fibrosis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC), which remains a major public health hazard worldwide.[1,2] Hepatitis B e antigen (HBeAg) status is usually used to stage CHB patients.[3] and it can be used as a predictive indicator of liver fibrosis in HBeAg positive patients.[4] A positive HBeAg status represents a high hepatitis B virus (HBV) replication rate and strong infectious state of CHB, which is one of the risk factors of liver fibrosis, LC and HCC.[5,6] According to the practice guidelines,[7,8] patients with a positive HBeAg status and hepatitis B virus deoxyribonucleic acid (HBV DNA) >4.3 log 10 IU/ml and alanine aminotransferase (ALT) >2 times the upper limit of normal (ULN) need antiviral treatment, while there is no clear antiviral treatment recommendation for HBeAg-positive patients with ALT ≤2-fold the ULN, unless they have significant liver fibrosis or inflammation. Previous studies[9,10] showed that 20% to 41% of these patients have obvious liver fibrosis but are easily ignored, since they usually have no obvious clinical symptoms. Liver biopsy is the gold standard for assessing fibrosis but is not widely accepted owing to its invasive nature, rare but potentially life-threatening complications, and prone to sampling errors.[11,12] Therefore, the development of non-invasive liver fibrosis prediction models could be favorable for this population.

Recently, transient elastography (TE) has widely been recognized for its ability to facilitate the non-invasive and repeatable assessment of liver fibrosis.[13,14] However, the diagnostic accuracy, which can be affected by the operator's skill, the lack of extensively validated cut-off values for specific stages of fibrosis, as well as the high cost of the equipment, may limit the clinical use of TE.[15,16] In addition, several non-invasive markers, such as the aspartate aminotransferase (AST)/ALT ratio index,[17] FIB-4 score,[18] Hui et al model,[19] Myers et al model,[20] PAPAS index,[21] and ATPI,[22] have also been developed. However, the biomarkers identified in these studies were mainly derived from patients with HBeAg-negative CHB, hepatitis C virus (HCV), or HCV/human immunodeficiency virus (HIV) coinfection; hence, they might not be suitable for CHB patients with other characteristics.[23,24]

In this study, we aimed to construct a non-invasive model based on routine serum markers to predict the liver fibrosis status of HBeAg-positive CHB patients with normal or slightly increased ALT, which may help doctors make appropriate antiviral treatment decisions, and may reduce the need of liver biopsy.

2. Methods

2.1. Patients

This cross-sectional study included antiviral treatment-naïve HBeAg-positive CHB patients with ALT levels ≤2-fold the ULN diagnosed at the Third People's Hospital of Shenzhen between January 2008 and December 2018. All patients met the following inclusion criteria: hepatitis B surface antigen (HBsAg)-positive; HBeAg-positive status for more than 6 months; simultaneous liver biochemistry and HBV serology assessments available, including HBsAg, HBeAg, and hepatitis B e antibody (anti-HBe) quantification; quantified HBV DNA; liver biopsy obtained before antiviral treatment; and a normal (≤40 IU/L) or slightly increased serum ALT level (≤2-times the ULN).[3] The exclusion criteria were as follows: liver comorbidities including HCV, hepatitis D virus (HDV), fatty liver disease, autoimmune liver diseases, drug-induced liver disease, decompensate liver disease, and HCC; and antiviral treatment in the past year.

This study was approved by the Medical Research Ethics Committee of the Third People's Hospital of Shenzhen in accordance with the principles of the Declaration of Helsinki (1975). All patients provided consent before participation according to the institutional guidelines.

2.2. Liver biopsy

Liver biopsy specimens (1.5 or 2.0 cm) were obtained percutaneously using a quick-cut16-gauge cutting needle under ultrasonographic guidance. Biopsy specimens with >7 portal areas were fixed with 10% formalin and paraffin-embedded for analysis. Hematoxylin-eosin and Masson's trichrome staining were performed for morphological evaluation and assessment of fibrosis, respectively. Liver fibrosis was assessed based on the METAVIR grade by liver pathologists who were blinded to the patients' clinical and viral results. The METAVIR fibrosis 4-grade staging was as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. F grades ≥2 was considered fibrosis.[25,26]

2.3. Laboratory tests

Blood samples were collected concurrently with liver biopsy for the quantification of serum HBsAg [the quantification of serum HBsAg (qHBsAg)] and HBeAg [the quantification of serum HBeAg (qHBeAg)], hepatitis B surface antibody, anti-HBe, antibodies to hepatitis C virus, antibodies to hepatitis D virus, and antibodies to human immunodeficiency virus antibodies using enzyme-linked immunosorbent assays. qHBsAg levels are expressed as IU/ml, and qHBeAg levels are expressed as Paul Erlich Institute Units/ml (PEIU/ml). DNA was isolated from plasma using the QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA) and stored at −20°C before to use. HBV DNA was assayed via quantitative polymerase chain reaction using commercial diagnostic kits (Da-an Gene Co., Guangzhou, China) with a lower limit of detection of 500 copies/ml. We converted HBV DNA units to IU/ml based on the international standard (1 IU/ml approximates 5.6 copies/ml).[8] Liver function was evaluated based on serum biochemical parameters, including ALT and AST, which were detected using commercial kits according to the manufacturer's instructions via an Olympus AU600 multifunctional biochemistry analyzer (Olympus, Japan). Serum ALT and AST levels were expressed as IU/L.

2.4. Statistical analysis

All analyses were performed using Empower Stats version 2.0 (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA, USA) and R statistical software version 3.5.3 (http://www.R-project.org, The R Foundation). Categorical variables are expressed as frequencies or percentages, and continuous variables are expressed as the mean ± standard deviation or median (quartile). The Chi-Squared or 2-sided Fisher exact test was used to identify significant differences in categorical data between groups. The Student *t* test or 1-way ANOVA test was used to compare the differences in parametric quantitative data. The Kruskal–Wallis or Mann–Whitney test was used to compare

nonparametric data. Serum HBsAg, HBeAg, and HBV DNA had highly skewed distributions; therefore, common logarithms were used in subsequent analyses. To screen for diagnostic factors in the training group, Spearman test and univariate logistic regression analysis were applied to examine the relationship between the fibrosis stage and each of the clinical parameters and routine biomarkers. We chose parameters with P values $<.05$ as independent variables. Multivariate logistic regression models were used to identify prognostic factors; the best predictive model was selected based on the minimal Akaike information criterion (AIC). To evaluate the models' diagnostic performance, receiver operating characteristic (ROC) analyses were performed to calculate the area under the curve (AUC). Diagnostic accuracy was assessed using the 95% confidence interval (CI), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The same methods were performed independently in the validation group. In addition, subgroup analyses of ALT and HBV DNA levels were performed using a stratified logistic regression model. To ensure robustness, we performed a sensitivity analysis using a calibration curve; $P<.05$ (2-sided) was considered statistically significant.

3. Results

3.1. Patient demographics and characteristics

Overall, 410 patients met the inclusion criteria and were naïve to antiviral treatment before undergoing liver biopsy at the Third People's Hospital of Shenzhen between January 2008 and December 2018. Thirty four patients were excluded because of HCV, HDV, or HIV coinfection (19), fatty liver disease (7), decompensated liver disease or HCC (5), or autoimmune liver diseases (3). Thirteen patients were excluded due to incomplete clinical data. A total of 363 patients were divided into a training group (n=266) and a validation group (n=97) via computer-generated randomization[28] (Fig. 1). Significant liver fibrosis was present (F $\geq$2) in 28.93% (105/363) of the patients. No significant differences were found in the patient characteristics between the training and validation groups (Table 1).

3.2. Associations between clinical parameters and liver fibrosis in the training group

Patients with significant liver fibrosis had significantly lower levels of qHBsAg, HBV DNA, and qHBeAg, and higher levels of serum ALT and AST, and were older than patients with mild fibrosis (all $P<.05$) (Fig. 2). According to univariate logistic regression analysis, all these clinical parameters were independent predictive variables of significant fibrosis (all $P<.05$) except sex ($P=.137$). Based on the Spearman test analysis, liver fibrosis grades were positively correlated with age ($r=0.294$) and serum AST ($r=0.258$); negatively correlated with qHBsAg ($r=-0.504$), qHBeAg ($r=-0.329$), and HBV DNA ($r=-0.309$); and showed a weak positive correlation with serum ALT levels ($r=0.154$) (all $P<.05$) (Table 2).

3.3. Construction and comparisons of non-invasive models to predict liver fibrosis

We selected age, qHBsAg, HBV DNA, qHBeAg, and serum ALT and AST as candidate parameters for our models because they significantly correlated with liver fibrosis. Based on multivariable logistic regression analysis, 2 types of non-invasive prediction models were established: full model (Model 1) and stepwise regression model (Model 2) (Table 3). The models were developed using the following formulas:

Model 1: logit (model value) = 6.09 + 0.09 × Age −2.36 × log10 (qHBsAg) −0.17 × log10 (HBV DNA) −0.52 × log10 (qHBeAg) + 0.02 × ALT + 0.03 × AST.

Model 2: logit (model value) = 5.67 + 0.08 × Age −2.44 × log10 (qHBsAg) −0.60 × log10 (qHBeAg) + 0.02 × ALT + 0.03 × AST.

Model 2 had the lowest AIC (Table 2 and Table 3). In the training group, the AUCs of the 2 models were 0.89 ($P>.05$);

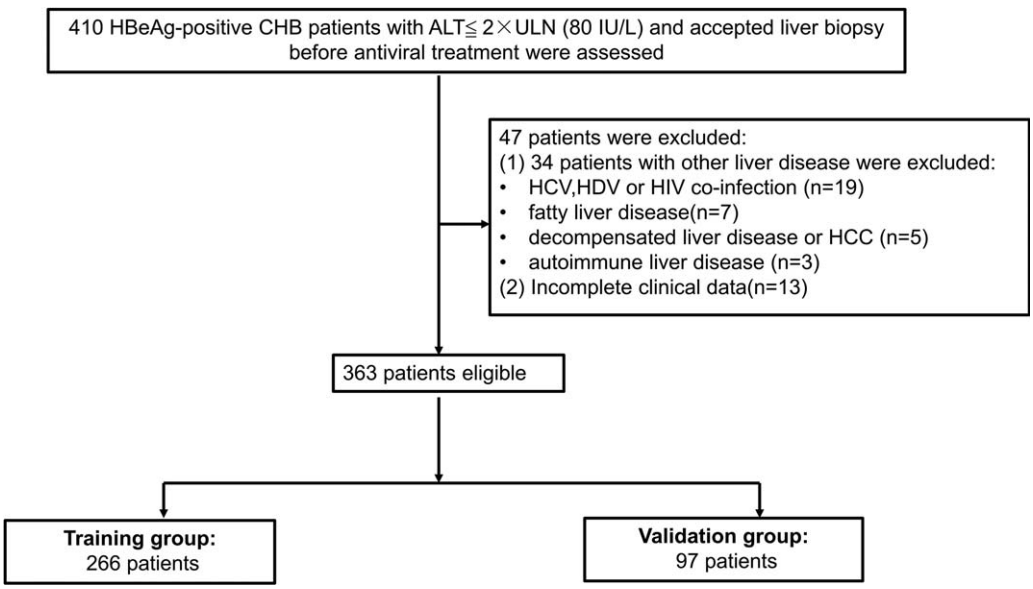

Figure 1. Flow diagram describing the selection of the study population. ALT = alanine aminotransferase, CHB = chronic hepatitis B infection, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HDV = hepatitis D virus, HIV = human immunodeficiency virus.

Table 1

Patient characteristics in training and validation groups.

Variables	All patients (n = 363)	Training group (n = 266)	Validation group (n = 97)	*P* value
Male	259 (71.35%)	188 (70.68%)	71 (73.20%)	.639
Age, yr	32.03 ± 7.32	31.95 ± 7.25	32.24 ± 7.53	.742
qHBsAg, IU/ml*	4.54 ± 0.73	4.55 ± 0.73	4.52 ± 0.73	.692
HBV DNA, IU/ml*	6.13 ± 1.43	6.13 ± 1.42	6.17 ± 1.48	.707
<4.3	40 (11.02%)	28 (10.53%)	12 (12.37%)	.619
≧4.3	323 (88.98%)	238 (89.47%)	85 (87.63%)	–
qHBeAg, PEI U/ml*	1.47 ± 0.69	1.44 ± 0.67	1.56 ± 0.73	.166
ALT, IU/L	44.36 ± 18.95	44.19 ± 18.91	44.82 ± 19.14	.777
≦1×ULN	161 (44.35%)	120 (45.11%)	41 (42.27%)	.629
>1×ULN and ≦2×ULN	202 (55.65%)	146 (54.89%)	56 (57.73%)	–
AST, IU/L	34.24 ± 13.25	34.17 ± 13.37	34.43 ± 12.99	.866
≦40	279 (76.86%)	203 (76.32%)	76 (78.35%)	.664
>40, ≦80	82 (22.59%)	61 (22.93%)	21 (21.65%)	–
>80	2 (0.55%)	2 (0.75%)	0 (0.00%)	–
Fibrosis stage				
F0/F1/F2/F3/F4	50/208/54/36/15	38/153/35/28/12	12/55/19/8/3	.576
Significant fibrosis (F≧2)	105 (28.93%)	75 (28.20%)	30 (30.93%)	.611

* Based on log10 transformed values; qHBsAg, the quantification of serum hepatitis B surface antigen; ALT = alanine aminotransferase, AST = aspartate aminotransferase, F = liver fibrosis stage, HBV = hepatitis B virus, qHBeAg = the quantification of serum hepatitis B–e antigen, ULN = the upper limit of normal.

Quantitative variables are expressed as mean ± standard deviation. Categorical variables are expressed as numbers (percentages). *P* values are for comparisons between the training and validation groups.

similar results were observed in the validation group (AUC 0.87 vs 0.86, $P > .05$) and all patients (AUCs 0.88, $P > .05$) (Fig. 3A-3C). Therefore, we considered Model 2 as the best option.

3.4. Diagnostic value and accuracy of the model

The ROC curve of Model 2 in predicting liver fibrosis in patients with HBeAg-positive CHB and ALT <2 ULN in the training group had an AUC of 0.89, which was significantly higher than that of qHBsAg (0.82), HBeAg (0.71), age (0.69), ALT (0.59), and AST (0.66) (all $P < .001$) (Fig. 3D, Sup 1, Supplemental Digital Content, http://links.lww.com/MD/G24). Similar results were observed for Model 2 in the validation group and in all patients (Fig. 3E-3F, Sup. 1, Supplemental Digital Content, http://links.lww.com/MD/G24).

Age, HBV DNA, and ALT should be taken into consideration when administering antiviral treatment.[3,7,8] We stratified all patients according to these 3 factors and assessed the predictive value of the model in different stratified stages. The AUC of Model 2 with patients aged ≥40 years was higher than that of

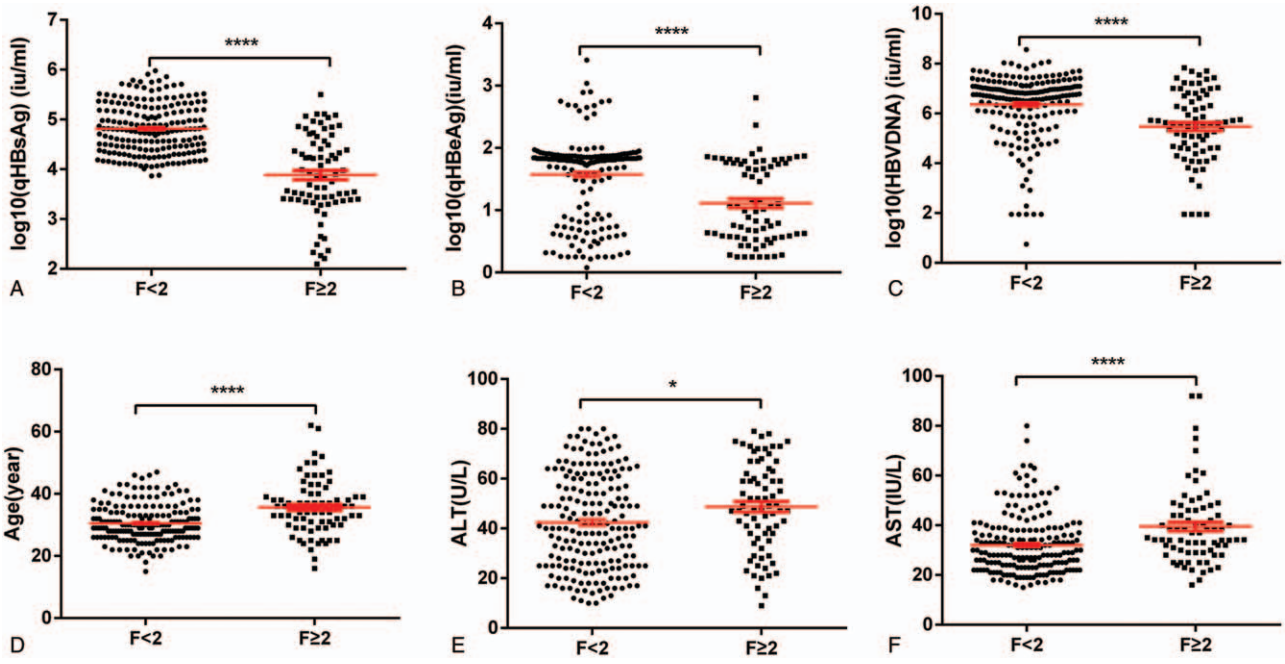

Figure 2. Comparisons of the various predicting parameters according to the liver fibrosis stages in patients with chronic hepatitis B in the training cohort. Comparisons of the levels of HBsAg (Fig. 2A), HBeAg (Fig. 2B), HBVDNA (Fig. 2C), Age (Fig. 2D), ALT (Fig. 2E), and AST (Fig. 2F) in different liver fibrosis stages. *$P < .05$; ****$P < .001$.

Table 2

Univariate and correlation analyses of clinical parameters potentially associated with liver fibrosis in the training group.

	Univariate analysis		Correlations analysis		
Variables	**OR (95% CI)**	***P* value**	**AIC**	***r***	***P* value**
Male	1.60 (0.86, 2.98)	.137	318.12	–	–
Age, yr	1.11 (1.06, 1.15)	<.001	293.50	0.294	.001
qHBsAg*	0.37 (0.29, 0.46)	<.001	221.78	–0.504	<.001
HBV DNA*	0.65 (0.54, 0.79)	<.001	300.22	–0.309	<.001
qHbeAg*	0.35 (0.23, 0.53)	<.001	294.94	–0.329	<.001
ALT	1.02 (1.00, 1.03)	.015	314.34	0.154	.014
AST	1.04 (1.02, 1.06)	<.001	304.49	0.258	<.001

* Based on log10 transformed values; ALT = alanine aminotransferase, AST = aspartate aminotransferase.

AIC = Akaike information criterion, CI = confidence interval, HBV = hepatitis B virus, OR = odds ratio, qHBeAg = the quantitative of hepatitis B-e antigen, qHBsAg = quantitative hepatitis B surface antigen, r = correlation coefficient.

Table 3

Construct the models based on multivariate logistic regression analysis of independent predictors in the training group.

	Model 1				Model 2			
Variables	**Estimate**	**OR**	**95% CI**	***P* value**	**Estimate**	**OR**	**95% CI**	***P* value**
(Intercept)	6.09	442.79	12.87–15236.15	<.001	5.67	290.54	9.02–9355.72	.001
Age	0.09	1.09	1.03–1.15	.002	0.08	1.09	1.03–1.14	.002
qHBsAg*	–2.36	0.09	0.04–0.20	<.001	–2.44	0.09	0.04–0.19	<.001
HBV DNA*	–0.17	0.85	0.66–1.10	.209	–	–	–	–
qHBeAg*	–0.52	0.60	0.33–1.08	.089	–0.60	0.55	0.31–0.98	.042
ALT	0.02	1.02	1.00–1.05	.077	0.02	1.02	1.00–1.05	.032
AST	0.03	1.03	1.00–1.06	.023	0.03	1.03	1.00–1.06	.027
AIC	195.99				187.69			

* Based on log10 transformed values; ALT = alanine aminotransferase, AST = aspartate aminotransferase.

AIC = Akaike information criterion, CI = confidence interval, HBV = hepatitis B virus, OR = odds ratio, qHBeAg = quantitative hepatitis B–e antigen, qHBsAg = quantitative hepatitis B surface antigen.

Model 1: Full model.

Logit (model value) = 6.09 + 0.09 × Age −2.36 × log10 (qHBsAg) −0.17 × log10 (HBVDNA) −0.52 × log10 (qHBeAg) + 0.02 × ALT + 0.03 × AST.

Model 2: Stepwise regression model.

Logit (model value) = 5.67 + 0.08 × Age −2.44 × log10 (qHBsAg) −0.60 × log10 (qHBeAg) + 0.02 × ALT + 0.03 × AST.

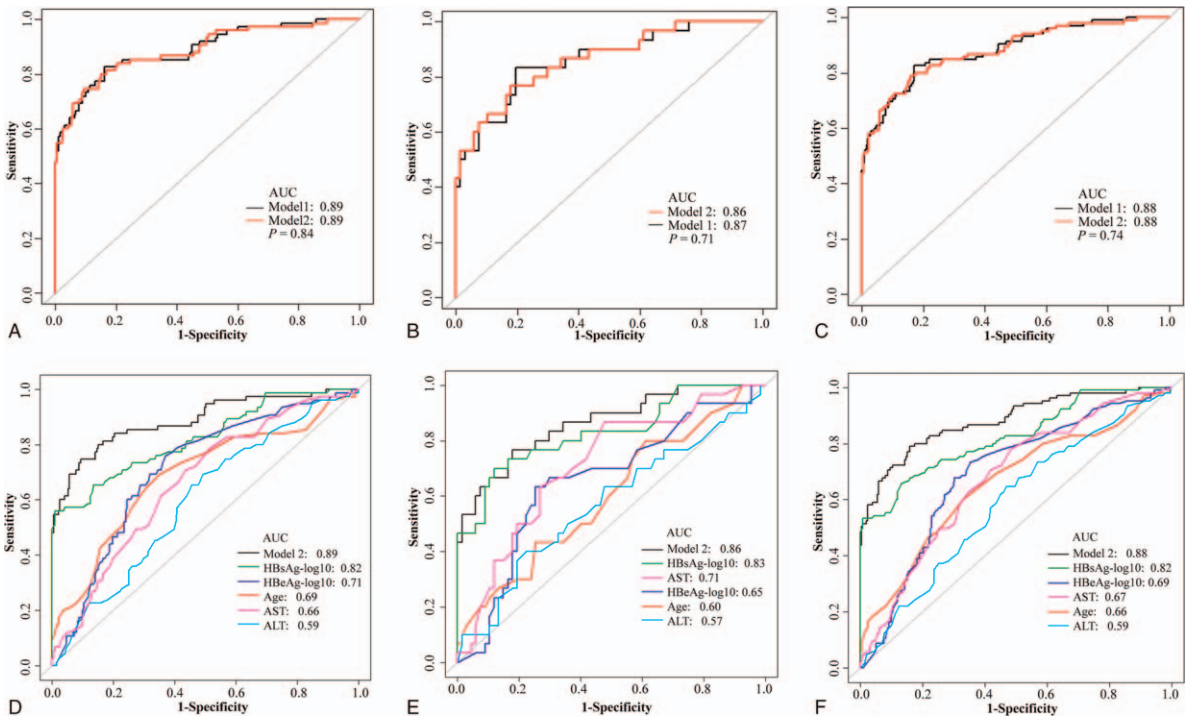

Figure 3. Comparisons of receiver operating characteristic (ROC) curves of different models and predicting parameters. Comparisons of AUC of the 2 models separately in training group, *P* = .84 (Fig. 3A), validation group, *P* = .71 (Fig. 3B), and total patients, *P* = .74 (Fig. 3C); Comparisons of AUC between different independent predicting parameters and Model 2 separately in training group (Fig. 3D), validation group (Fig. 3E) and all CHB patients (Fig. 3F).

Table 4

Hierarchical analysis of Model 2 in predicting liver fibrosis in HBeAg-positive patients with alanine aminotransferase normal or slightly increased.

	All patients	Mild set (n)	Significant set (n)	AUC	95% CI	Best threshold	Sp	Se
All patients	363	258	105	0.88	0.84–0.92	−1.17	0.83	0.83
Age, yr								
<40	311	232	79	0.88	0.82–0.92	−1.19	0.88	0.75
≥40	52	26	26	0.96	0.82–0.99	0.17	0.96	0.77
HBV DNA* IU/ml								
<4.3	40	20	20	0.82	0.67–0.97	−0.16	0.95	0.70
≥4.3	323	238	85	0.89	0.85–0.93	−1.19	0.85	0.80
ALT, IU/L								
<40	161	118	33	0.89	0.82–0.96	−1.77	0.82	0.81
≥40	202	140	72	0.87	0.82–0.93	−0.28	0.91	0.69
ALT<40 IU/L and HBV DNA >7 log10 IU/ml								
	50	44	6	0.81	0.66–0.97	−3.48	0.61	1.00

* Based on log10 transformed values; ALT = alanine aminotransferase, AUC = area under the curve of the receiver operating characteristic, HBV = hepatitis B virus, Se = sensitivity, Sp = specificity.

patients aged <40 years (0.96 vs 0.88). The ROC curve of Model 2 showed that patients with log10 (HBV DNA) ≥4.3 IU/ml had a larger AUC than patients with log10 (HBV DNA) <4.3 IU/ml (0.89 vs 0.82). The AUC of Model 2 was relatively stable after stratification by ALT <40U/L or ALT ≥40U/L (0.89 vs 0.87). According to the practice guideline of EASL (2017)[8]: HBeAg-positive patients with ALT normal and HBV DNA >7 log10 IU/ml (107 IU/ml) are defined as HBeAg-positive HBV infection patients, the AUC of Model 2 in this population was 0.81 (95% CI: 0.66–0.97), indicating that Model 2 is also applicable to HBeAg-positive CHB infection patients. (Table 4).

3.5. Clinical application of the model

Two points were selected as cut-off values for predicting liver fibrosis based on the ROC analysis of Model 2 in the training group: a low cut-off point (−2.61) was chosen to obtain a sensitivity of at least 95%, and a high cut-off point (0.25) was chosen to provide specificity of at least 95%[29] (Table 5). In the training group, 96% (95/99) of patients with a model value ≤−2.61 had mild liver fibrosis (NPV: 0.96, specificity: 0.50, sensitivity: 0.95); 46 out of 56 patients (82%) with a model value ≥0.25 had significant liver fibrosis (PPV: 0.82, specificity: 0.95, sensitivity: 0.61); only 25 of 111 patients with model values between the 2 cut-off points (23%) had significant liver fibrosis.

Applying these cutoffs to the validation group (Table 5), 36 of 39 patients with a model value <−2.61 (92%) were found to have mild liver fibrosis (NPV: 0.92, specificity: 0.54, sensitivity: 0.90); a total of 16 out of 20 patients (80%) with a model value >0.25 had significant liver fibrosis (PPV, 0.84; specificity, 0.96; sensitivity, 0.53); about 29%(11/38) of patients with model values between the 2 cut-off points had significant liver fibrosis.

The AUC of Model 2 in all patients was 0.88. Using the 2 cut-off points (−2.6 and 0.25), all patients were divided into 3 groups: About 95% of patients (131/138) with model value ≤−2.61 had mild liver fibrosis. These patients were not required undergo antiviral therapied or liver biopsy; Most of patients with model value ≥0.25 (82%) had significant liver fibrosis and these patients could be recommended for antiviral treatment without liver biopsy; For patients with a model value between −2.61 and 0.25, liver biopsy was still required. Applying this model, 59% of patients (214/363) could be determined with their liver fibrosis status without liver biopsy and given corresponding treatment recommendations; 149 patients were recommended to undergo liver biopsy for accurate diagnosis, which may help to reduce the need for liver biopsy. (Fig. 4, Table 5).

Table 5

Cut-off values within the derived model for classifying liver fibrosis.

	Cut-off	Total (n)	Mild set (n)	Significant set (n)	Sp (95% CI)	Se (95% CI)	PPV (95% CI)	NPV (95% CI)
Training group		266	191	75				
	≦−2.61	99	95	4	0.50 (0.42–0.57)	0.95 (0.86–0.98)	0.43 (0.35–0.50)	0.96 (0.89–0.99)
	−2.61< and <0.25	111	86	25				
	≧0.25	56	10	46	0.95 (0.90–0.97)	0.61 (0.49–0.72)	0.82 (0.69–0.91)	0.86 (0.80–0.90)
Validation group		97	67	30				
	≦−2.61	39	36	3	0.54 (0.41–0.66)	0.90 (0.72–0.97)	0.47 (0.34–0.60)	0.92 (0.78–0.98)
	−2.61< and <0.25	38	27	11				
	≧0.25	20	4	16	0.96 (0.87–0.99)	0.53 (0.35–0.71)	0.84 (0.60–0.96)	0.82 (0.71–0.89)
Total		363	258	105				
	≦−2.61	138	131	7	0.51 (0.45–0.57)	0.93 (0.86–0.97)	0.44 (0.37–0.50)	0.95 (0.89–0.98)
	−2.61< and <0.25	149	113	36				
	≧0.25	76	14	62	0.95 (0.91–0.97)	0.59 (0.49–0.68)	0.82 (0.71–0.89)	0.85 (0.80–0.89)

NPV = negative predictive value, PPV = positive predictive value, Se = sensitivity, Sp = specificity.

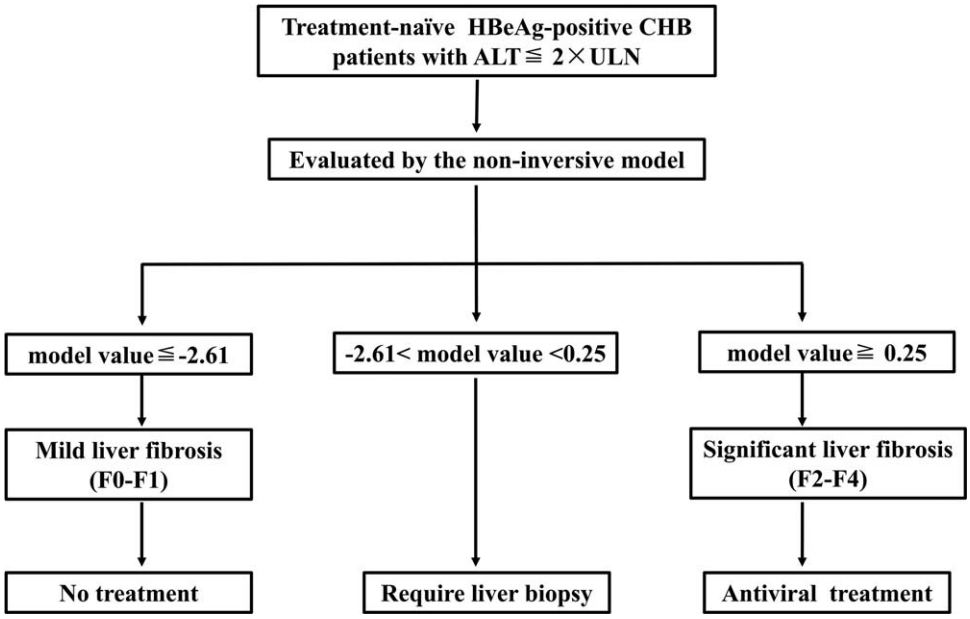

Figure 4. Algorithm for the application of the model to predict liver fibrosis in HBeAg-positive patients with normal or slightly increased alanine aminotransferase.

4. Discussion

The precise staging of liver fibrosis in patients with HBeAg-positive CHB who have ALT levels ≤2-fold the ULN is important for making antiviral therapy decisions.[3,7,8] Considering that liver biopsy and imaging are not widely suitable for patients in primary clinical practice, non-invasive diagnosis of liver fibrosis is a very attractive strategy. Our study aimed to develop a non-invasive model for accurately staging liver fibrosis for these CHB patients. In this study, Model 2 was selected as the best model based on the AIC value. The AUC for Model 2 was stable in training group (0.89), validation group (0.86), and all the patients (0.88), and the AUC improved to 0.96 for patients over 40 years of age. Using the cutoffs of −2.61 and 0.25, Model 2 could successfully predict liver fibrosis and the eligibility for antiviral treatment in 59% of all patients, which may reduce the need for liver biopsy.

Age is an effective predictor of significant fibrosis in patients with CHB, especially in patients over 40 years of age.[30] Therefore, we included age in our model. Moreover, we found that patients with significant fibrosis had lower levels of qHBsAg, HBV DNA, and qHBeAg than patients with mild fibrosis; this result is consistent with previous studies.[31–33] A possible explanation is that enhanced immune clearance of HBV viral or intracellular block of HBV markers secretion may be involved in such a process.[21,34,35] We found that HBV DNA negatively correlated with liver fibrosis stage [odds ratio = 0.65, $r = -0.309$, all $P < .001$] through univariate logistic regression analysis and Spearman test analysis (Table 2). However, multivariate logistic regression analysis in Model 1 showed that there was no significant difference in the correlation between HBV DNA and liver fibrosis ($P = .209$) (Table 3). To find out the factors that may affect the statistical results, we used the collinearity variance inflation factor (VIF) analysis. A VIF ≥10, indicated strong multicollinearity between explanatory variables. The results showed that the VIF values of all parameters were <10, which indicated that the collinearity interference between parameters and liver fibrosis could be eliminated (sup. 2, Supplemental Digital Content, http://links.lww.com/MD/G25). Interaction between HBV DNA and HBsAg ($P = .015$) (sup. 3, Supplemental Digital Content, http://links.lww.com/MD/G26) was observed using interaction analysis, indicating that the correlation between HBV DNA and liver fibrosis may be affected by HBsAg. Although the mechanism of the interaction between the 2 parameters still is still unclear, several studies have suggested that serum HBV DNA levels in HBeAg-positive patients are positively correlated with liver inflammation, but not with liver fibrosis.[36–38] Therefore, we removed HBV DNA from our final model.

Increased fibrosis increases the release of ALT and AST from sick or damaged cells;[36,39] which may explain the positive correlations between liver fibrosis and serum ALT and AST levels in this study. We also found that the association between fibrosis and AST ($r = 0.258$) was stronger than that between fibrosis and ALT ($r = 0.154$); this may be due to liver fibrosis-associated mitochondrial injury or increased release of AST relative to ALT.[29,40] Although these conventional serum markers are commonly tested during the course of the disease, they may not accurately predict the severity of liver fibrosis. The model we constructed with these parameters using multivariate logistic regression analysis could greatly improve the prediction of liver fibrosis.

In this study, the non-invasive model that we developed has several unique characteristics. First, it only uses routine serum markers (qHBsAg, qHBeAg, ALT, and AST) and age, which are easy and inexpensive to obtain. Therefore, our model is especially suitable for primary medical facilities. Second, our model can dynamically monitor and assess liver fibrosis status in patients with CHB who are widespread and easily overlooked in antiviral treatment decisions. Third, the model has a good predictive value for liver fibrosis in CHB patients, and the results showed excellent repeatability.

Our study also had several limitations. First, this was a cross-sectional study, and the model was constructed and randomly validated at the same center. Whether the model can be used to assess treatment response still needs a prospective cohort study to be conducted, and multicenter studies are needed to validate its

reliability. Second, we defined the normal ALT level as <40 IU/L, despite a recent recommendation stating that a normal ALT level should be considered as <35 U/L for men and <25 U/L for women.[7] As only 19% of patients in this study had ALT values within these lower ranges, we were unable to use them for our analyses. Lastly, we did not perform HBV genotype sequencing, which has not been a routine clinical test in most primary medical facilities; however, we aimed to build a non-invasive model that is suitable for most medical institutions, and many hospitals do not perform the HBV genotype test. Despite these limitations, our data provide some necessary information on the histological characteristics of livers in Chinese patients with CHB.

5. Conclusions

In summary, the non-invasive model developed in this study serves as an independent index to evaluate the liver fibrosis of HBeAg-positive CHB patients with ALT levels ≤2-fold the ULN. Which can help clinicians make appropriate antiviral treatment decisions and may reduce the need for liver biopsy. Multicenter verification of the model's stability and feasibility is necessary.

Acknowledgments

The authors would like to thank all the participants, especially the donors, in this study. We also want to thank the doctors and nurses at the Third People's Hospital of Shenzhen for helping to recruit and follow up with the donors in this study.

Author contributions

Conceptualization: Guoliang Zhang, Shiping Hu and Qing He.
Data curation: Ling Li.
Formal analysis: Yongan Ye.
Funding acquisition: Shiping Hu.
Investigation: Shuyan Liu, Yaya Liu and Qiyuan Tang.
Methodology: Ling Li, Juanjuan Zhang, Guohui Xiao.
Project administration: Shiping Hu.
Resources: Guoliang Zhang, Qing He.
Supervision: Xuejiao Liao, Qing He.
Validation: Ling Li, Yun Ran.
Visualization: Jian Lu.
Writing – original draft: Ling Li.
Writing – review & editing: Ling Li, Yongan Ye.

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