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Original Article

Exploration and verification of liver fibrosis non-invasive diagnostic model in patients with chronic hepatitis B

Danying Cheng, Yingying Zhao, Shuang Luo, Peng Wang, Fengshui Wang, Xiaomei Wang, Weini Ou, Ying Duan, Huichun Xing, Jun Cheng

Liver Disease Center of Beijing Ditan Hospital, Capital Medical University, Beijing, China

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Abstract: Objective: This study aimed to establish a new non-invasive diagnostic model for liver fibrosis in Chinese patients with chronic hepatitis B (CHB) and to evaluate its diagnostic accuracy. Methods: This was a prospective study on the liver histology of 240 CHB patients. Patients were divided into 2 groups: 120 patients were included for the establishment of diagnostic model (modeling group) and remaining patients for the validation. Results: A new non-invasive diagnostic model for liver fibrosis contained two parameters: P = -0.32+0.568 * LnFS (kPa)-0.035 * LnPLT (10^9/L), and liver fibrosis index was calculated as $e^P/(1+e^P)$. The area under the receiver operating characteristic curve (AUROC) for significant fibrosis was 0.934 (95% CI = 0.865, 1.0) in modeling group (n = 120) and 0.891 ((95% CI = 0.811, 0.971) in validation group (n = 120). When the model value was <0.58, the negative predictive value was 76%, the sensitivity was 100% for significant fibrosis, the specificity was 95%, the positive predictive value was 100% and the negative predictive value was 76% with the model index >0.88. When the data of all patients were analyzed with Forns Index, Fibro index and Fibrotest model, the AUROC was 0.756, 0.705 and 0.742, respectively, which were lower than in the new model. Conclusion: The newly established non-invasive diagnostic model is good for the diagnosis of liver fibrosis in CHB patients, especially in those with significant liver fibrosis. Fibroscan has a higher diagnostic accuracy in liver fibrosis caused by CHB and can be used as an independent predictor.

Keywords: Liver fibrosis, serum markers, Fibroscan, non-invasive model

Introduction

Liver fibrosis may be found in patients with almost all kinds of liver diseases, such as chronic viral hepatitis, alcohol liver disease, metabolic liver disease, autoimmunity liver disease and others and may progress into irreversible liver cirrhosis if timely and appropriate treatments are not applied. Nowadays, the methods used for the diagnosis of liver cirrhosis mainly include histopathologic, radiological, physical and serologic examinations. Liver biopsy has long been used as the gold standard in the assessment of fibrosis in patients with chronic liver diseases (CLD), but it is invasive and its accuracy is related to the number and size of samples [1-3]. In addition, there is still inter-observer variation in the pathological examination [4]. Thus, it is necessary to develop a non-invasive method for the diagnosis of liver fibrosis with a high sensitivity and a high specificity. Fibroscan (FS) has been widely used in the past decade due to its non-invasiveness, repeatability and low cost. It depends on the sound wave to identify fibrotic liver tissues [5].

When the serological parameters are detected simultaneously, FS can improve the accuracy of diagnosis of liver fibrosis as reported in previous studies. Currently, there are two kinds of models used for the non-invasive diagnosis of liver fibrosis: one is consisted of clinical routine detection, such as APRI, FIB-4 and Forns, and the other is made up of models for liver cirrhosis, like Fibrotest (FT), FibroSpect II, Europe liver cirrhosis group model (ELFG), FibroMeter, Hepascore, Shanghai Liver Cirrhosis Group Model (SLFG), FibroIndex, Fibro-pro and others, which are tested by clinicopathologically with better accuracy, sensitivity and specificity [6-15]. Which non-invasive diagnostic model is better for the diagnosis of liver fibrosis in Chinese patients with chronic hepatitis B (CHB) and whether combined use of these models is

needed are still unclear. In this study, the serological parameters and liver elasticity were detected for the establishment of a new model for the non-invasive diagnosis of liver fibrosis in Chinese patients with CHB.

Materials and methods

Patients

This was a prospective study on the liver histology of 240 patients with CHB who underwent liver biopsy from September 2011 to March 2012 in Beijing Ditan Hospital. These patients were randomly divided into 2 groups: modeling group (n = 120) and validation group (n = 120). The inclusion and exclusion criteria were as follows:

Inclusion criteria

Liver fibrosis was diagnosed according to the diagnostic criteria in the Guideline for Prevention and Treatment of Chronic Hepatitis B in China; patients were aged between 18 years and 65 years; patients were positive for serum hepatitis B surface antigen and hepatitis B virus DNA for at least 6 months; alanine aminotransferase (ALT) was between the upper limit of normal (ULN) to 10 times of ULN; informed consent was obtained before study.

Exclusion criteria

Patients were not positive for serum anti-HAV IgM, anti-HCV, anti-HDV, anti-HEV and anti-HIV positive; patients had no other liver diseases such as autoimmune hepatitis, hepatolenticular degeneration, primary biliary cirrhosis, alcoholic liver disease and drug-induced liver disease; patients had other pre-existing severe diseases such as cancer, severe cardiopulmonary disease and unstable diabetes; patients were pregnant or breast-feeding women; patients got a cold or had tonsillitis and other infectious disease in the one month before study; patients participated in other clinical research(es) in 6 months before this study; patients were suspected to have primary liver cancer or alpha-fetoprotein >100 ng/ml; patients had ascites, serious cholestasis (serum total bilirubin higher than 10 times of ULN) or hepatic decompensation.

Baseline characteristics

The gender, age, admission number, contact information, history of alcohol drinking, family history of hepatitis B and previous treatments were recorded.

Blood collection

All blood samples were collected within 1 week after liver biopsy, kept at room temperature for 1 h and centrifuged at 2000 rpm for 20 min. Then, the serum was collected and stored at -20°C.

Detection of parameters

All conventional examinations including blood routine test and detection of prothrombin activity (PTA), liver function, kidney function and blood lipids were conducted in the Clinical Laboratory of Beijing Ditan Hospital. Hyaluronic acid (HA), amino-terminal peptide of type III procollagen (PIIIP), tissue inhibitors of metalloproteinases (TIMPs), α_2-macroglobulin (α_2 MG or AMG) and haptoglobin (Hp) were also measured by enzyme-linked immunosorbent assay with kits from Beijing Ya Anda Biotechnology Co., Ltd. (Dong Songs Biological).

Liver biopsy and pathological examination

Percutaneous transhepatic biopsy was done by ultrasound guidance with 18 G Bard needle (1-Second Method). All the specimens collected by liver biopsy were fixed in 10% formalin, embedded in paraffin, followed by HE staining and Masson Trichrome staining for further pathological assessment, which was done independently by two pathologists in Department of Pathology of Beijing Ditan Hospital. The liver inflammation was graded (A0-A3) according to the inflammation in the hepatic lobules, the portal area and other areas, and the liver fibrosis was staged (F0-F4) according to the Metavir scoring system. Stage F0-F1 was defined as non-significant liver fibrosis and stage F2-F4 as significant liver fibrosis.

Detection of liver elasticity

Liver stiffness was measured one week before liver biopsy in all the patients and represented by elasticity (kpa). FibroScan ultrasonic diagnostic apparatus was purchased from the French Echosens company. In brief, patients were placed in a supine position with the right arm on the right posterior quarter of the head. Detection was conducted at the midaxillary line and the xiphoid crossline intersection of inter-

Table 1. Correlation of liver inflammation grade and fibrosis stage in CHB patients

Fibrosis Stage (F)	Inflammation Grade (A)			
	0	1	2	3
0	0	3	0	0
1	0	165	14	0
2	0	8	26	0
3	0	4	11	4
4	0	0	5	0

costal space or at the site of liver puncture determined by ultrasound. The probe was vertical to the skin, and the "time pattern" and A-scan signal graph were observed. When the A-scan signal became approximately linear, probe was nudged until the pressure displayed area was green. A least 10 successful figures were captured in each subject and the median echo was calculated. The success rate was greater than 60%.

Statistical analysis

Statistical analysis was done with SPSS version 19.0. Continuous variables are described as mean ± standard deviation (SD) or quartiles, and compared with *t*-test or one way analysis of variance; categorical variables were described as frequency or ratio and compared with chi-square test or Fisher exact test. A value of $P<0.05$ was considered statistically significant.

The influence of all the factors on the dependent variable was quantitatively analyzed by logistic regression analysis and a diagnostic model was established. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of factors in the diagnosis of liver fibrosis were calculated. Then, receiver operating characteristic (ROC) curve analysis was performed and the area under the ROC curve was calculated.

Results

General characteristics

Of 240 patients, there were 156 males (64.97%) and 84 females (35.03%), and the mean age was 36.86±11.22 years (range: 18 years to 65 years). As to the liver inflammation degree, stage A1 was found in 180 patients (75.00%), stage A2 in 56 (23.33%) and stage A3 in 4 (1.67%). As to liver fibrosis degree, stage F0 was found in 3 patients (1.25%), stage F1 in 179 (74.58%), stage F2 in 34 (14.17%), stage F3 in 19 (7.92%) and stage F4 in 5 (2.08%). Non-significant liver fibrosis (stage F0-F1) was found in 182 patients (75.83%) and significant liver fibrosis (stage F2-F4) in 58 (24.17%) (**Table 1**).

Correlation between liver inflammation grade and fibrosis stage in CHB patients

The correlation between liver inflammation grade and fibrosis stage was evaluated in CHB patients of this study. Results showed that there was a significant positive correlation between liver inflammation grade and fibrosis stage (correlation coefficient: 0.732; $P<0.01$), indicating that the more severe the liver inflammation is, the more severe the liver fibrosis is (**Table 1**).

Exploration and verification of liver fibrosis non-invasive diagnosis model

The 240 patients were randomly divided into modeling group and verification group at a ratio of 1:1, and comparisons were done with t-test or one way analysis of variance. Results showed that there were no significant differences in the baseline characteristics between two groups (**Table 2**).

In the modeling group, cases were divided into 2 groups according to the degree of liver fibrosis (F0-F1 subgroup and F2-F4 subgroup). Performed forward regression analysis was performed to evaluate the influence of factors on the dependent variables with the Logistic regression method. Significant influence was found in 4 parameters including age, PLT, ALB and FS (**Table 3**). Finally, PLT and FS were determined as the independent predictors. The multiple regression equation was established according to the independent predictors and the regression coefficient. The exponential model of the mathematical formula was converted to 0-1. The calculation formula of the model is: P = -0.32+0.568 * LnFS (Kpa)-0.035 * LnPLT (10^9/L), liver fibrosis index = $e^P/(1+e^P)$. Then, the ROC curves were drawn, and the diagnostic accuracy of the model was analyzed when the model was taken. Univariate logistic regression analysis showed age, PLT, ALB and

Table 2. General characteristics of patients in modeling group and verification group

Parameters	Modeling group (n = 120)	Verification group (n = 120)	*P* value
Age (yr)	37.44±11.74	35.63±10.07	>0.05
Gender (male)	82 (68.33%)	74 (57.89%)	>0.05
WBC (10^9/L)	5.75 (2.8-11.42)	5.36 (2.78-10.7)	>0.05
RBC (g/L)	4.80 (3.09-6.65)	4.78 (3.79-6.61)	>0.05
HB (g/L)	147.38 (106-188)	147.34 (97-188.1)	>0.05
PLT (10^9/L)	188.10 (82.1-349)	182.51 (57.4-270.1)	>0.05
PT (s)	12.33 (9.9-15.3)	12.02 (9.8-14)	>0.05
PTA (%)	87.47 (66-127)	87.40 (75-131)	>0.05
INR (s)	1.05 (0.8-1.24)	1.02 (0.88-1.18)	>0.05
ALT (U/L)	51.89 (10.1-302.6)	63.93 (8.9-316.6)	>0.05
AST (U/L)	34.71 (13.7-138.2)	39.34 (13.2-316.8)	>0.05
TBIL (μmol/L)	16.09 (6.2-106.9)	15.09 (7.1-43.4)	>0.05
DBIL (μmol/L)	5.95 (1.3-78.1)	4.65 (1.6-14.6)	>0.05
ALB (g/L)	44.982 (29.6-53.3)	45.98 (30.4-56)	>0.05
GGT (U/L)	50.13 (7.2-1500.1)	45.33 (5.8-428.3)	>0.05
ALP (U/L)	75.34 (39.3-127.1)	83.11 (39.2-215)	>0.05
CR (μmol/L)	64.90 (38-86.3)	65.17 (40-92)	>0.05
TC (mmol/L)	4.44 (2.86-7.35)	4.59 (2.73-6.94)	>0.05
ApoA1 (g/L)	1.24 (0.4-1.89)	1.30 (0.51-2.12)	>0.05
HA (μg/L)	219.05 (137.64-318.73)	221.07 (137.64-305.87)	<0.05
PIIIP (ng/mL)	89.08 (42.65-150.65)	89.70 (34.62-155.95)	>0.05
TIMPs (μg/L)	205.25 (225.27-289.74)	211.32 (131.29-284.01)	>0.05
AMG (μg/ml)	6.19 (2.63-9.87)	6.07 (3.1-8.27)	>0.05
HPT (μg/ml)	227.08 (33.4-454.09)	233.42 (83.06-442.91)	>0.05
FS (Kpa)	9.70 (2.5-29.6)	8.80 (4.3-26.1)	>0.05
Inflammation grade			>0.05
A0	0	0	
A1	87 (72.5%)	93 (77.5%)	
A2	29 (24.17%)	27 (22.5%)	
A3	4 (3.33%)	0	
Fibrosis stage			>0.05
F0	2 (1.67%)	1 (0.83%)	
F1	85 (70.83%)	94 (78.33%)	
Non-significant liver fibrosis	87 (72.5%)	95 (79.17%)	
F2	17 (14.17%)	17 (14.17%)	
F3	12 (10%)	7 (5.83%)	
F4	4 (3.33%)	1 (0.83%)	
Significant liver fibrosis	33 (27.5%)	25 (20.83%)	

Notes: Continuous variables were transformed to natural logarithms to optimize the distribution normality. Continuous variables were analyzed with one way analysis of variance and expressed as mean ± SD or median (25% quantile, 75% quantile); categorical variables were tested with chi-square test and expressed as n (%).

FS were closely related to the liver fibrosis (**Table 3**). However, in the multivariate regression analysis, only PLT and FS were found to be the independent predictors of liver fibrosis. The multivariate regression equation was P = -0.32+0.568 * LnFS (Kpa)-0.035 * LnPLT (10^9/L) and the liver fibrosis index was $e^P/(1+e^P)$. Then, ROC was delineated, and the diagnostic accuracy was determined. The working curve of the model is shown in **Figure 1**.

The critical values of the model were 0.58 and 0.88 when the sensitivity was 95% and specificity was 95%, respectively. When the model

Table 3. Parameters used for the establishment of diagnostic model for liver fibrosis according to the degree of liver fibrosis (F2-F4 and F0-F1)

Parameters	Modeling group (n = 120)	F0-F1 (n = 87)	F2-F4 (n = 33)	*P* value
Age (yr)	37.44±11.74	35.70±11.34	42.03±11.73	<0.05
Gender (M)	82 (68.33%)	57 (65.52%)	25 (75.75%)	>0.05
WBC (10^9/L)	5.75±1.53	5.91±1.60	5.34±1.28	>0.05
RBC (g/L)	4.80±0.60	4.86±0.61	4.64±0.55	>0.05
Hb (g/L)	147.38±15.66	147.43±16.64	147.25±16.60	>0.05
PLT (10^9/L)	189.61±55.89	206.47±47.77	145.69±51.99	<0.05
PT (s)	12.33±0.95	12.24±0.86	12.57±1.15	>0.05
PTA (%)	87.47±12.58	88.09±11.90	85.79±14.33	>0.05
INR (s)	1.05±0.10	1.04±0.09	1.06±0.11	>0.05
ALT (U/L)	51.89±47.30	46.35±33.73	67.73±72.02	>0.05
AST (U/L)	34.71±22.21	31.51±16.06	43.86±32.91	>0.05
TBIL (μmol/L)	16.09±11.83	16.47±13.28	15.02±6.21	>0.05
DBIL (μmol/L)	5.95±8.07	6.26±9.30	5.05±2.24	>0.05
ALB (g/L)	44.98±4.54	45.66±4.30	43.04±4.70	<0.05
GGT (U/L)	50.13±159.82	53.88±188.72	40.83±29.70	>0.05
ALP (U/L)	75.34±20.77	76.68±20.37	72.00±21.78	>0.05
CR (μmol/L)	64.90±12.06	64.47±12.33	66.02±11.50	>0.05
TC (mmol/L)	4.44±0.95	4.59±1.00	4.07±0.74	>0.05
ApoA1 (g/L)	1.24±0.34	1.25±0.38	1.21±0.26	>0.05
HA (μg/L)	219.05±46.33	219.27±47.22	218.47±44.12	>0.05
PIIIP (ng/mL)	89.08±23.11	88.56±23.00	90.43±23.67	>0.05
TIMPs (μg/L)	205.25±37.00	207.32±38.54	199.78±32.53	>0.05
AMG (μg/ml)	6.19±1.31	6.14±1.19	6.35±1.57	>0.05
HPT (μg/ml)	227.08±63.55	229.14±65.29	221.66±59.36	>0.05
FS (Kpa)	9.70±6.45	6.79±2.46	16.71±7.63	<0.05
Inflammation grade				<0.05
A0	0	0	0	
A1	87 (72.5%)	79 (90.8%)	8 (24.24%)	
A2	29 (24.17%)	8 (9.20%)	21 (63.63%)	
A3	4 (3.33%)	0	4 (12.12%)	

index was <0.58, 48 patients were included (40.0% of all the patients), with the sensitivity of 95%, specificity of 27%, PPV of 37% and NPV of 76% for non-significant liver fibrosis, and 28% of 48 patients definitely had no significant liver fibrosis; when the model index was >0.88, 14 patients were included (11.7% of all the patients), with the sensitivity of 100%, specificity of 95%, PPV of 100% and NPV of 76% for significant liver fibrosis, and all the 14 patients had definitely significant liver fibrosis.

First, the *P* value was calculated after substituting the data of patients in the verification group in the new model. Then, the liver fibrosis index was calculated. The ROC curve was delineated and the AUC was calculated as to be 0.891 for significant fibrosis in the verification group (**Figure 2**).

Analysis was made with the critical value of the verification group. When the model index was <0.37, 10 patients were qualified (11.7% of the total patients) with the sensitivity of 95%, specificity of 31%, PPV of 33% and NPV of 72% for non-significant liver fibrosis, and 26% of 10 patients had definitely no significant liver fibrosis; when the model index was >0.82, 8 patients were qualified (8.3% of the total patients) with the sensitivity of 100%, specificity of 95%, PPV of 100% and NPV of 61% for significant liver fibrosis, and all the 8 patients had definitely significant liver fibrosis.

Comparison with other existing models

To compare the accuracy of this model to that of other models, the related data of 240 patients were input into other models, and results were compared. Forns model includes four parameters: PLT, GGT, Age and TC and the equation was Forns Index = 7.811-3.131 × Ln (PLT (10^9/L)) +0.781 × Ln (GGT (U/L)) +3.467 × Ln (Age [yr]) -0.014 × (TC [mg/dL]). FibroIndex model includes four parameters: Age, PLT, GGT and HA. The equation was: FibroIndex = 10 × eD/(1+eD); D = -6.29+1.678 × Ln (age [yr]) -1.786 × Ln (PLT [10^9/L]) +1.177 × Ln (GGT [U/L]) +1.019 × Ln (HA [ng/m1]). Fibrotest was a non-invasive diagnostic model established with direct serum parameters in patients with chronic hepatitis C. This model

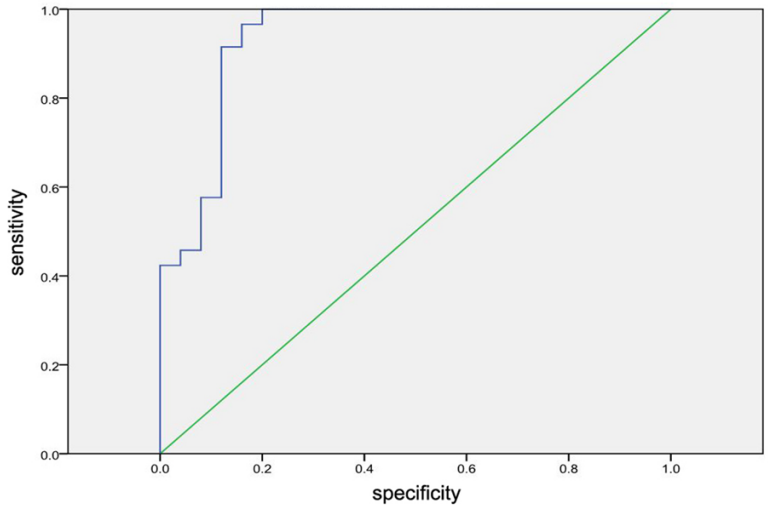

Figure 1. ROC Curve of Modeling Group.

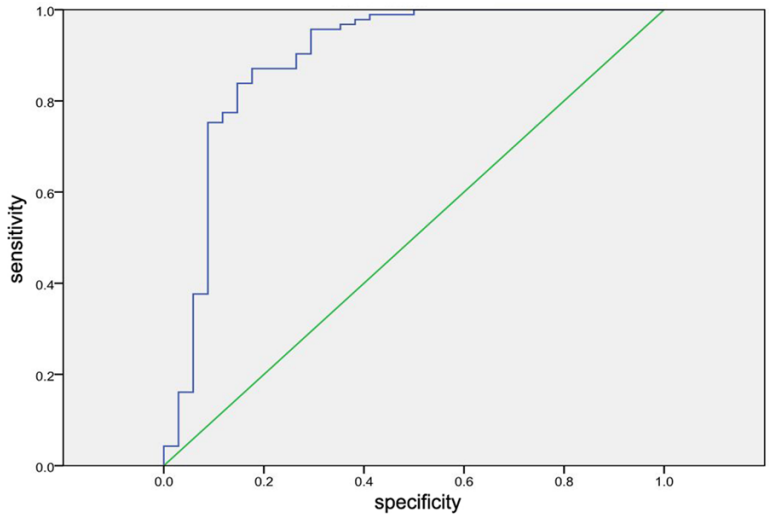

Figure 2. ROC in Verification Group.

includes a total of 7 parameters: A2M, HA, GGT, TB apoA1, age and gender. The equation was FT = 4.467 × logα_2M (g/L) -1.357+1.017 × log-HAP (ng/m1) GGT (U/L) +0.0281 × log × Age (yr) +1.737 × log the BIL (μmol/L) -1.184 × ApoA1 (g/L) +0.301 × Gender (female = 0, male = 1) -5.540 [2, 3, 5-10]. The comparisons were done with ROC curve (**Figure 3**). Results showed that the new model in our study had a largest AUC.

Discussion

Currently, pathological examination after liver biopsy is still the gold standard for the diagnosis of liver fibrosis [11]. However, this method is invasive and has high cost, high risk for bleeding and other complications as well as poor compliance, which significantly limit its wide application in clinical practice. Moreover, the location and size of samples collected by biopsy may affect the diagnostic accuracy, especially when the distribution of liver inflammation and fibrosis is uneven. Thus, it is imperative to develop an ideal non-invasive method for the diagnosis of liver fibrosis. Along with the introduction of Fibroscan as a new technique for the diagnosis of liver fibrosis, increasing attention has been paid to the detection of liver elasticity. Although it is a non-invasive, painless and quick method, the diagnostic accuracy is probably affected by obesity, ascite, activity of liver inflammation and other factors [5, 12-15]. The diagnostic models established with multiple parameters are highly valued in recent years for their better performance in distinguishing significant and non-significant liver fibrosis than any of the single-parameter model [16-20]. However, most of models are established in hepatitis C and non-B hepatitis patients, and their effectiveness for patients with hepatitis B is needed to be further validated. There are some models that have been established or validate in Chinese studies, but they are not widely applied yet [21-23].

In this study, we established a new diagnostic model based on serum parameters and FS in 120 patients with CHB and this model was further validated in 120 CHB patients. With two parameters, PLT and FS, this model had a higher AUROC as well as higher NPV and PPV at both sides of the diagnostic critical value. The AUROC in verification group was 0.89. When

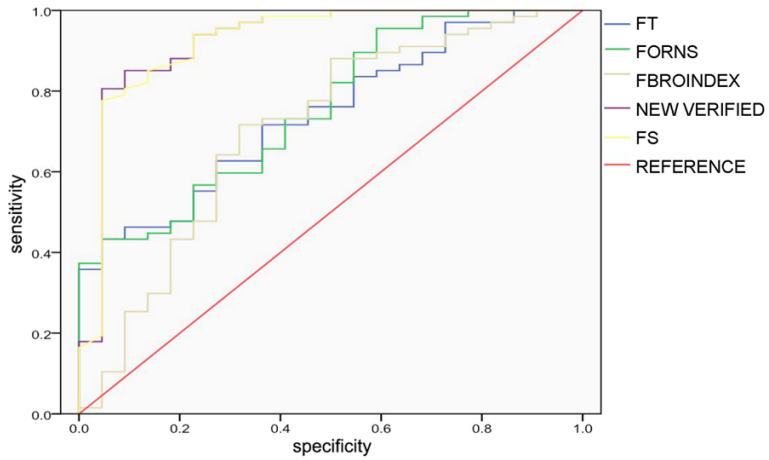

Figure 3. ROC Curves of 3 Existing Models, Fibroscan and our Model.

the model index was <0.37, the sensitivity was 95% and NPV was 72% for non-significant liver fibrosis; when the model index was >0.82, the sensitivity was 100%, specificity was 95% and PPV was 100% for significant liver fibrosis, showing higher stability and better diagnostic efficiency. This model is better to determine the significant/non-significant liver fibrosis and parameters used in this model are easily to measure in clinical practice. Thus, it will have a favorable prospect of application, especially in patients with significant liver fibrosis. Of note, its efficacy still needs validation in studies with large sample size.

Our results also showed that FibroScan, an independent predictor of liver fibrosis, has a high diagnostic accuracy in CHB patients, which was even higher than the serological model in determining significant/non-significant liver fibrosis. The new model established with the combined use of serum parameters and FS had a much better diagnostic accuracy than other serological models did. These results show that the FS-based models are better than serological models in the diagnosis of liver fibrosis in terms of the accuracy, which further confirms the diagnostic value of FibroScan in liver fibrosis of CHB patients.

However, in the present study, some imaging parameters such as spleen size and portal width are not employed for the establishment of diagnostic model. Whether these parameters are valuable for the non-invasive diagnosis of liver fibrosis is warrant to be confirmed in future studies.

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Disclosure of conflict of interest

None.

Address correspondence to: Huichun Xing and Jun Cheng, Liver Disease Center of Beijing Ditan Hospital, Capital Medical University, Beijing, China. E-mail: xinghc2016@163.com (HCX); jun.cheng.ditan@gmail.com (JC)

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