### Title

A Novel Noninvasive Program for Staging Liver Fibrosis in Untreated Patients With Chronic Hepatitis B




### Journal

Clinical and Translational Gastroenterology, 10(5)

### ISSN

2155-384X



### Authors

Chen, Yan
Wang, Yongji
Chen, Yongping
et al.



### Publication Date

2019-05-22

### DOI

10.14309/ctg.0000000000000033

# A Novel Noninvasive Program for Staging Liver Fibrosis in Untreated Patients With Chronic Hepatitis B

Yan Chen, MD, PhD[1], Yongji Wang, PhD[2], Yongping Chen, MD[3], Zujiang Yu, MD, PhD[4], Xiaoling Chi, MD[5], Ke-Qin Hu, MD[6], Qin Li, MD[7], Lin Tan, MD, PhD[8], Dedong Xiang, MD[9], Qinghua Shang, MD[10], Chunliang Lei, MD[11], Liang Chen, MD, PhD[12], Xiaoyu Hu, MD, PhD[13], Jing Wang, MD[14], Huabao Liu, MD[15], Wei Lu, MD, PhD[16], Weilai Chi, PhD[17], Zheng Dong, MD[1], Xiaodong Wang, PhD[3], Zhiqin Li, MD[4], Huanming Xiao, MD, PhD[5], Da Chen, MD[6], Wenlin Bai, MD[1], Changjiang Zhang, MD[9], Guangming Xiao, MD[11], Xun Qi, MD[12], Jing Chen, MD[13], Li Zhou, MD[15], Huiwei Sun, BA[1], Minghua Deng, PhD[17], Xiaolong Qi, PhD[18], Zheng Zhang, MD, PhD[1], Xingshun Qi, MD[19] and Yongping Yang, MD[1]

OBJECTIVES: **Chronic hepatitis B (CHB) can progress into liver fibrosis and cirrhosis with poor outcomes. Early and accurate diagnosis of liver fibrosis/cirrhosis is important to guide the preventive strategy of their related complications.**

METHODS: **A Chinese multicenter cross-sectional study was conducted to develop and validate a novel noninvasive program for staging liver fibrosis in untreated patients with CHB. Liver histology was evaluated independently by 2 pathologists. The alanine aminotransferase ratio, Hepascore, and aspartate aminotransferase to platelet index values were calculated. Liver stiffness measurement (LSM) and diameter of the spleen were measured. Logistic regression with $\ell_1$ penalty of regression coefficients was used to select the optimal predictors. The diagnostic accuracy for the stage of liver fibrosis was assessed by the area under the receiver operator characteristic curve with 95% confidence interval (CI).**

RESULTS: **A total of 1,200 patients with CHB were included, of whom 800 and 400 were in training and validation sets, respectively. LSM, platelets, age, hyaluronic acid, and diameter of the spleen were the top 5 predictors associated with any stage of liver fibrosis and integrated into a novel noninvasive program, named as the Chin-CHB score. The area under the receiver operator characteristic curve of the Chin-CHB score was 0.893 (95% CI: 0.77–0.92) for diagnosing significant fibrosis, 0.897 (95% CI: 0.85–0.95) for advanced fibrosis, and 0.909 (95% CI: 0.87–0.95) for cirrhosis. The diagnostic performance of the Chin-CHB score was similar between training and validation sets. The Chin-CHB score had better diagnostic performance than aspartate aminotransferase to platelet index, alanine aminotransferase ratio, LSM alone, and Hepascore for diagnosing any stage of liver fibrosis.**

CONCLUSIONS: **The Chin-CHB score had good diagnostic performance for any stage of liver fibrosis.**

**SUPPLEMENTARY MATERIAL** accompanies this paper at http://links.lww.com/CTG/A31, http://links.lww.com/CTG/A32, http://links.lww.com/CTG/A33, and http://links.lww.com/CTG/A34

*Clinical and Translational Gastroenterology 2019;10:e-00033. https://doi.org/10.14309/ctg.0000000000000033*

[1]Center of Therapeutic Research for Liver Cancer, Beijing 302 Hospital, Beijing, China; [2]Department of Health Statistics, the Fourth Military Medical University, Xi'an, Shanxi Province, China; [3]Department of Infectious and Liver Diseases, Liver Research Center, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, China; [4]Department of Infectious Disease, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China; [5]Traditional Chinese Medicine Hospital of Guangdong Province, Guangdong Province, China; [6]Division of Gastroenterology and Hepatology, University of California, Irvine, School of Medicine, Orange, California, USA; [7]Fuzhou Infectious Diseases Hospital, Fuzhou, Fujian Province, China; [8]Liver Disease Department, Fuyang 2nd People's Hospital, Fuyang, Anhui Province, China; [9]Department of Infectious Diseases, Southwest Hospital, the Third Military Medical University, Chongqing, China; [10]Therapeutic Center for Liver Disease, the 88th Hospital of PLA, Taian, Shandong Province, China; [11]Guangzhou 8th People's Hospital, Guangzhou, Guangdong Province, China; [12]Department of Hepatic Diseases, Shanghai Public Health Clinical Center, Shanghai, China; [13]National Integrative Medicine Clinical Base for Infectious Diseases/Department of Infectious Diseases, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, China; [14]Affiliated Traditional Chinese Medicine Hospital of Luzhou Medical University, Luzhou, Sichuan Province, China; [15]Traditional Chinese Medicine Hospital of Chongqing, Chongqing, China; [16]Tianjin Second People's Hospital, Tianjin Institute of Hepatology, Tianjin, China; [17]Center for Quantitative Biology, Peking University, Beijing, China; [18]CHESS, The Fifth Department of General Surgery, The First Hospital of Lanzhou University, Lanzhou, China; [19]Liver Cirrhosis Study Group, Department of Gastroenterology, General Hospital of Shenyang Military Area, Shenyang, Liaoning Province, China. **Correspondence:** Yongping Yang, MD. E-mail: yongpingyang@hotmail.com. Zheng Zhang, MD, PhD. E-mail: zhangzheng1975@aliyun.com. Ke-Qin Hu, MD. E-mail: kqhu@uci.edu.

**Received October 19, 2018; accepted February 22, 2019; published online April 25, 2019**

## INTRODUCTION

Chronic hepatitis B (CHB) causes liver fibrosis and then liver cirrhosis and hepatocellular carcinoma, which can result in life-threatening complications, such as gastrointestinal bleeding and liver failure (1,2). In the algorithm for management of CHB, early and accurate diagnosis of liver fibrosis/cirrhosis is particularly important to guide the preventive strategy of their related complications (3). Currently, liver biopsy remains the gold standard approach for staging liver fibrosis, but is often limited by its invasiveness, poor acceptance, sampling variability, and complications (4,5). In addition, repeated biopsy is often unacceptable for dynamic assessment of progression and regression of liver fibrosis. In this setting, the physicians have paid more and more attention to noninvasive assessment of liver fibrosis by physical examinations, routine biochemical and hematological tests, and surrogate serum fibrosis markers (6,7). Several noninvasive models have been developed, such as the FibroTest (8), aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR) (9), AST to platelet index (APRI) (10), and Hepascore (11), etc. However, their diagnostic accuracy is not satisfactory. For example, the evidence from meta-analyses of 37 articles suggested that the APRI had moderate diagnostic performance for significant fibrosis, advanced fibrosis, and cirrhosis in patients with CHB with summary areas under the receiver operating characteristic curve of less than 0.8 (12). Some components included in these models, such as haptoglobin, α2-macroglobulin, and apolipoprotein A1, are not routinely available in clinical practice. It has been reported that liver stiffness measurement (LSM) may have a higher diagnostic accuracy for assessment of advanced liver fibrosis and cirrhosis than serum biomarkers in patients with CHB (13,14), but its usefulness is often questioned in some specific population (i.e., obesity and ascites) (15). In addition, the performance of noninvasive markers for diagnosing middle stages of fibrosis is reduced (16).

We have conducted a Chinese multicenter cross-sectional study involving 1,200 patients with CHB to develop and validate a noninvasive program (i.e., Chin-CHB score) for accurately differentiating liver fibrosis stage by stage based on 30 routinely available parameters.

## METHODS

### Study design

The study protocol was approved by the ethical committees of all 14 participating institutions, including Beijing 302nd Hospital, the First Affiliated Hospital of Wenzhou Medical University, the First Affiliated Hospital of Zhengzhou University, Fuzhou Infectious Diseases Hospital, Traditional Chinese Medicine Hospital of Guangdong Province, Fuyang 2nd People's Hospital, Southwest Hospital of the Third Military Medical University, the 88th Hospital of PLA, Guangzhou 8th People's Hospital, Shanghai Public Health Clinical Center, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Traditional Chinese Medicine Hospital of Chongqing, and Tianjin 2nd People's Hospital. Written informed consent was obtained from all patients. Data included in this multicenter cross-sectional study were from an ongoing randomized controlled trial regarding regression of hepatitis B virus (HBV)-related liver fibrosis (NCT01965418).

Inclusion criteria were as follows: (i) men or women aged 18–65 years, positive hepatitis B surface antigen (HBsAg) for at least 6 months; (ii) as for HBeAg-positive CHB, HBV DNA ≥20,000 IU/mL and ALT ≥2 × upper normal limit, as for HBeAg-negative CHB, HBV DNA ≥2,000 IU/mL and ALT ≥2 × upper normal limit, or clinically compensated cirrhosis with detectable serum HBV DNA regardless of the ALT level; (iii) a baseline liver biopsy specimen obtained within 4 weeks before enrollment; and (iv) no antiviral or antifibrotic therapy within 6 months before enrollment. Exclusion criteria were as follows: (i) coinfection with other virus hepatitis or chronic liver diseases; (ii) liver biopsy was inadequate for grading and/or staging; (iii) one or more variables were missing; and (iv) decompensated cirrhosis or history of any concurrent malignancy.

### Laboratory tests

Fasting blood samples (10 mL) were collected and processed independently at each center. Major laboratory parameters included complete blood counts, urea, creatinine, bilirubin, γ-glutamyl transpeptidase, alkaline phosphatase (ALP), albumin, albumin/globulin ratio, AST, ALT, prothrombin index, α-fetoprotein, triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, apolipoprotein A1, haptoglobin, hyaluronic acid (HA), propeptide of type III procollagen, α2-macroglobulin, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and HBV DNA level.

The AAR value was calculated by the formula : (AST/ALT ratio).

The Hepascore value was calculated by the formula:

$$\frac{\exp[-4.185818-(0.0249\times \text{age})+(0.7464\times \text{sex})+(1.0039\times \alpha2\text{-macroglobulin})+(0.0302\times \text{HA})+(0.0691\times \text{TBIL})-(0.0012\times \text{GGT})]}{1+\exp[-4.185818-(0.0249\times \text{age})+(0.7464\times \text{sex})+(1.0039\times \alpha2\text{-macroglobulin})+(0.0302\times \text{HA})+(0.0691\times \text{TBIL})-(0.0012\times \text{GGT})]},$$

with age provided in years, male = 1, female = 0, α2-macroglobulin in g/L, HA in μg/L, TBIL in μmol/L, and GGT in U/L.

The APRI value was calculated by the formula: AST level (/upper normal limit)/platelet (PLT)$(10^9/\text{L})\times 100$.

### LSM

LSM with XL probe on the FibroScan 502 Touch device (Echosens, France) was performed at each center by an experienced operator blinded for patient data according to the instructions provided by the manufacturer. The LSM can be valid if the following criteria are fulfilled according to the European Association for Study of Liver-Asociacion Latinoamericana para el Estudio del Higado Clinical Practice Guideline (14): (i) the number of valid shots should be at least 10; (ii) the success rate (a ratio of the number of valid shots to the total number of shots)

should be above 60%; and (iii) an interquartile range (IQR), which reflects the variability of measurements, should be less than 30% of the median value (M) of LSM (IQR/M $\leq$ 0.30%). LSM result is expressed in kilopascals (kPa), which ranges from 1.5 to 75 kPa with a normal value of 5 kPa.

### Spleen diameter measurement

Diameter of the spleen (dSpleen), which refers to the maximum spleen bipolar diameter, was estimated on ultrasounds by an experienced operator at each center. dSpleen is expressed in millimeters (mm).

### Liver histology

Ultrasound-guided liver biopsy was performed. A Quick-cut needle or Menghini needle (16 G) was used for biopsy. Specimens were fixed with 10% formalin, routinely embedded in paraffin, and stained with hematoxylin/eosin and Masson trichrome. As for the required liver histological specimens, the minimal length should be 2.0 cm with at least 11 portal tracts. All liver histological specimens from 14 sites were reviewed independently by 2 pathologists, who were blinded to the clinical and laboratory data. Liver fibrosis was staged using the METAVIR score as follows (17): F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and few septa; F3 = numerous septa without cirrhosis; and F4 = cirrhosis. Significant fibrosis was defined as a METAVIR score of 2 or higher, advanced fibrosis as a METAVIR score of 3 or higher, and cirrhosis as a METAVIR score of 4. Necroinflammatory activity was graded on a 4-point scale, A0 = no activity; A1 = mild activity; A2 = moderate activity; and A3 = severe activity.

### Statistical analyses

Quantitative variables are expressed as medians (IQR) and compared by the nonparametric test, whereas categorical variables are expressed as numbers (percentage) and compared by the Fisher exact test.

Logistic regression with $\ell_1$ penalty of regression coefficients was used to select the optimal predictors associated with various stages of liver fibrosis. The accuracy of diagnosing the stage of liver fibrosis was assessed by the area under the receiver operator characteristic curve (AUC). Cross-validation was adopted to select variables, and tuning the shrinkage parameter, lambda, 5-fold cross-validations were repeated 10 times with different splits for the data. The penalized logistic regression was fitted on training sets and subsequently used to predict the binary labels of the validation sets. And we selected the lambdas with the best prediction AUC on validation set for each time, which could shrink some coefficients to zeros. Ordinal logistic regression was used to build an ordered regression model for the each end point (significant fibrosis, advanced fibrosis, and cirrhosis) with the selected optimal variables (18). After fitting the model, a linear combination of the optimal variables with cumulative link model was built, which is named as the Chin-CHB score with the values of LSM provided in kPa, PLT in $10^9$/L, age in years, dSpleen in mm, and HA in μg/L.

$$S = 2.35 \times ln(LS) - 1.22 \times ln(PLT) + 1.48 \times ln(Age) + 0.34 \times ln(HA) + 0.89 \times ln(dSpleen)$$

S represents the stage of liver fibrosis. S1, S2, and S3 represent significant fibrosis, advanced fibrosis, and cirrhosis, respectively. S is the sharing measurement of 3 classification problems, and $\theta_i$ is the intercept, which corresponds to each class.

$$logit(P(S \geq S_i | Data)) = S - \theta_i, i = 1, 2, 3$$

The conditional cumulative probabilities are as follows:

| Stage of fibrosis | Cumulative probability | Formula |
|---|---|---|
| Significant fibrosis | $P(Sign.\|Data) = \frac{exp(I_1)}{1+exp(I_1)}$ | $I_1 = S - 6.71$ |
| Advanced fibrosis | $P(Adva.\|Data) = \frac{exp(I_2)}{1+exp(I_2)}$ | $I_2 = S - 9.52$ |
| Cirrhosis | $P(Cirr.\|Data) = \frac{exp(I_3)}{1+exp(I_3)}$ | $I_3 = S - 10.93$ |

Stage of fibrosis resulted from an "incomplete measurement" of the Chin-CHB score, where one only determines the interval into which S fell:

**Table 1. Baseline characteristics**

| Variables | Training set (n = 800) | Validation set (n = 400) | *P* value |
|---|---|---|---|
| Age (median, IQR) (yr) | 41 (34–47) | 40 (33–47) | 0.122 |
| Female (n, %) | 246 (31) | 130 (32) | 0.553 |
| Body mass index (median, IQR) | 23 (21–27) | 23 (21–28) | 0.82 |
| Drinker (n, %) | 86 (11) | 41 (10) | 0.843 |
| Smoker (n, %) | 119 (15) | 58 (14) | 0.931 |
| Quantitative HBsAg (median, IQR) ($10^3$ IU/mL) | 2.75 (0.97–9.01) | 3.13 (1.25–11.6) | 0.042 |
| HBeAg positive (n, %) | 417 (52) | 232 (58) | 0.057 |
| HBV DNA (median, IQR) ($10^5$ IU/mL) | 6.54 (0.43–294) | 13.1 (0.72–493) | 0.083 |
| HBV DNA 3–5 × $10^5$ IU/mL (n, %) | 41 (5.1) | 21 (5.2) | 1 |
| HBV DNA >5 × $10^5$ IU/mL (n, %) | 421 (53) | 240 (60) | 0.018 |
| Diameter of the spleen (median, IQR) (mm) | 36 (32–39) | 35 (32–38) | 0.524 |

**Table 1. (*continued*)**

| Variables | Training set (n = 800) | Validation set (n = 400) | *P* value |
|---|---|---|---|
| Diameter of the spleen >35 mm (n, %) | 468 (58) | 231 (58) | 0.852 |
| Diameter of the spleen (median, IQR) (mm) | 110 (101–119) | 108 (101–117) | 0.047 |
| Liver stiffness (median, IQR) (Kpa) | 8.51 (5.60–14.3) | 8.22 (5.52–13.8) | 0.494 |
| White blood cell (median, IQR) ($\times 10^9$/L) | 5.31 (4.41–6.40) | 5.52 (4.42–6.91) | 0.041 |
| Platelet counts (median, IQR) ($\times 10^9$/L) | 166 (125–204) | 176 (137–213) | 0.02 |
| Platelet counts $<100 \times 10^9$/L (n, %) | 92 (12) | 51 (13) | 0.592 |
| Prothrombin time (median, IQR) (seconds) | 12 (11–14) | 12 (11–14) | 0.607 |
| Fibrinogen (median, IQR) (g/L) | 2.21 (1.92–2.60) | 2.30 (2.01–2.60) | 0.332 |
| Aspartate aminotransferase (median, IQR) (IU/L) | 46 (26–85) | 43 (26–85) | 0.596 |
| Alanine aminotransferase (median, IQR) (IU/L) | 55 (32–89.5) | 55 (33–107.5) | 0.157 |
| Alanine aminotransferase $<1 \times$ ULN (n, %) | 136 (17) | 64 (16) | 0.782 |
| Alanine aminotransferase 1–2 $\times$ ULN (n, %) | 239 (30) | 112 (28) | 0.545 |
| Alanine aminotransferase $>2 \times$ ULN (n, %) | 425 (53) | 224 (56) | 0.338 |
| Albumin (median, IQR) (g/L) | 43 (40–46) | 43 (40–46) | 0.126 |
| Globulin (median, IQR) (g/L) | 37 (24–65) | 35 (26–60) | 0.53 |
| Total bilirubin (median, IQR) (μmol/L) | 30 (26–32) | 29 (26–33) | 0.92 |
| Blood urea nitrogen (median, IQR) (mmol/L) | 4.41 (3.80–5.51) | 4.30 (3.71–5.40) | 0.597 |
| Creatinine (median, IQR) (μmol/L) | 72 (61–83) | 71 (60–83) | 0.616 |
| Alkaline phosphatase (median, IQR) (U/L) | 89 (70–107) | 87 (70–104) | 0.148 |
| γ-glutamyl transferase (median, IQR) (U/L) | 33 (20–58) | 30 (20–54) | 0.258 |
| Adenosine deaminase (median, IQR) (U/L) | 16 (12–24) | 15 (12–22) | 0.022 |
| Cholinesterase (median, IQR) ($10^3$ U/L) | 6,916 (5,699–8,071) | 7,134 (5,934–8,378) | 0.055 |
| Haptoglobin (median, IQR) (g/L) | 428 (197–696) | 473 (257–728) | 0.074 |
| α2-macroglobulin (median, IQR) (g/L) | 2,310 (1888–2,832) | 2,320 (1888–2,815) | 0.987 |
| Hyaluronic acid (median, IQR) (μg/L) | 60 (39–109) | 58 (41–111) | 0.888 |
| Propeptide of type III procollagen (median, IQR) (μg/L) | 7 (6–10) | 7 (6–10) | 0.77 |
| METAVIR fibrosis (n, %) | | | 0.468 |
| F0/F1 | 28/131 (3.5/16.375) | 12/71 (3.0/17.75) | |
| F2 | 314 (39.3) | 142 (35.5) | |
| F3 | 130 (16.3) | 62 (15.5) | |
| F4 | 197 (24.6) | 113 (28.8) | |
| Significant fibrosis (F2–F4) | 641 (80.1) | 317 (79.2) | 0.76 |
| Advanced fibrosis (F3–F4) | 327 (40.9) | 175 (43.8) | 0.352 |
| Cirrhosis (F4) | 197 (24.6) | 113 (28.8) | 0.184 |
| METAVIR activity (n, %) | | | 0.508 |
| A0 | 196 (24.5) | 94 (23.5) | |
| A1 | 360 (45.0) | 190 (47.5) | |
| A2 | 149 (18.6) | 79 (19.8) | |
| A3 | 95 (11.9) | 37 (9.20) | |
| Significant activity (A2 and A3) (n, %) | 244 (30.5) | 116 (29) | 0.64 |
| Concurrent fatty liver (n, %) | 159 (19.8) | 85 (21.25) | 0.577 |

Drinker was defined as habitual alcohol consumption indicated by drinking alcohol ≥4 d per wk for ≥1 yr. Smoker was defined as current smoking or a history of smoking.
IQR, interquartile range.

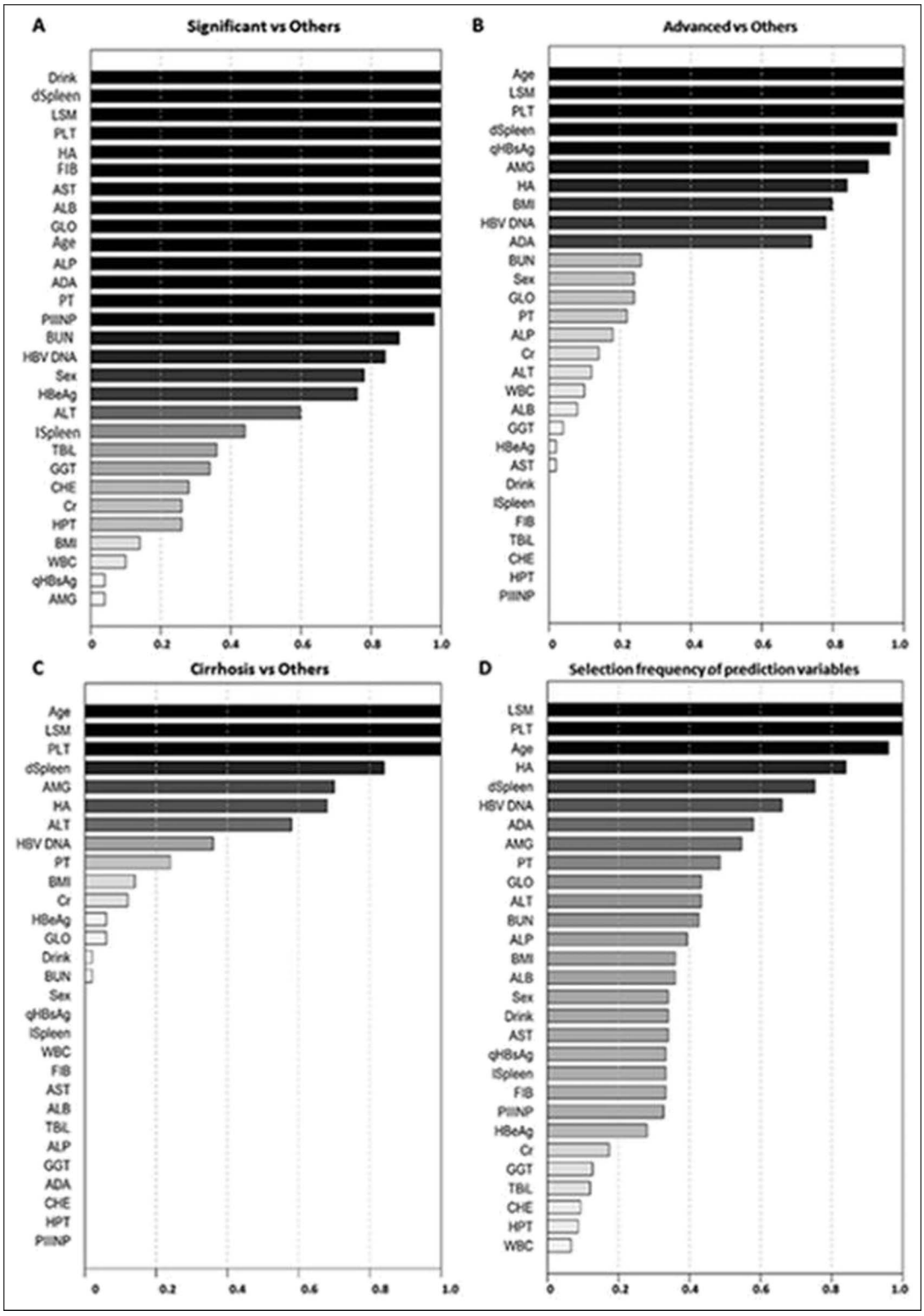


**Figure 1.** Predictors for staging liver fibrosis that are ranked according to the statistical significance. (**a**) Predictors for distinguishing significant fibrosis. (**b**) Predictors for distinguishing advanced fibrosis. (**c**) Predictors for distinguishing cirrhosis. (**d**) Selection frequency of predictors for distinguishing any stage of liver fibrosis. Notes: X-axis refers to the frequency of the most stable variables for staging liver fibrosis, which were selected in the model. In the training set, we performed penalized logistic regression analyses 100 times. Every time, we would select some optimal variables. If a variable was selected 100 times, the frequency would be 100%. If a variable was selected 50 times, the frequency would be 50%. ADA, adenosine deaminase; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMG, a2-macroglobulin; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CHB, chronic hepatitis B; dSpleen, diameter of the spleen; FIB, fibrinogen; GGT, γ-glutamyltransferase; GLO, globulin; HA, hyaluronic acid; HPT, haptoglobin; LSM, liver stiffness measurement; PIINP, propeptide of type III procollage; PT prothrombin time; TBL, total bilirubin; WBC, white blood cell.

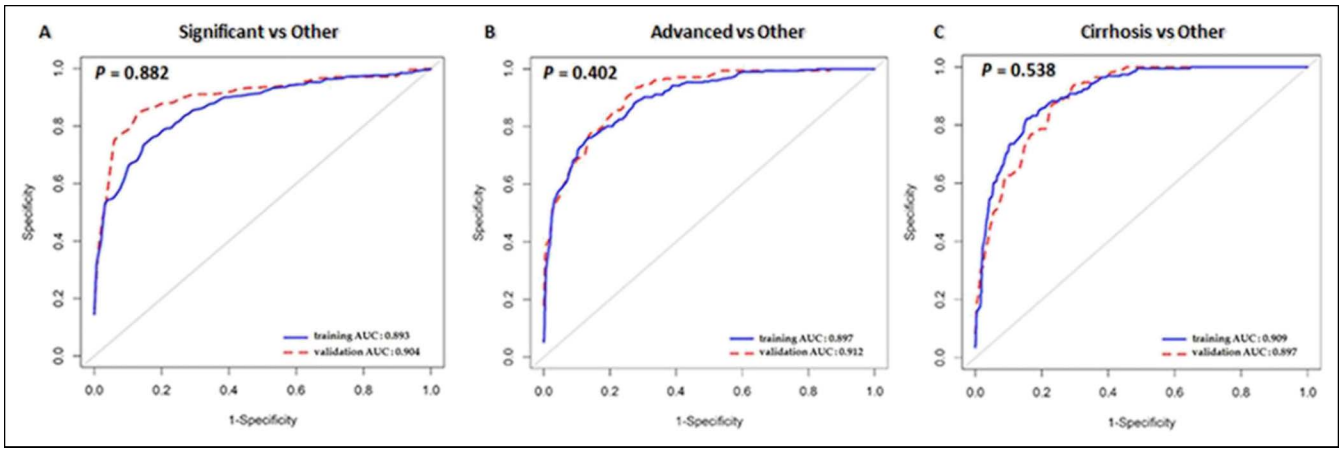


**Figure 2.** ROC analyses of the Chin-CHB score in both training and validation sets. (**a**) Distinguishing significant fibrosis. (**b**) Distinguishing advanced fibrosis. (**c**) Distinguishing cirrhosis. AUC, area under the receiver operator characteristic curve; CHB, chronic hepatitis B; ROC, receiver operator. Characteristic curve.

$$fibrosis\ \ stage = \begin{cases} mild\ \ or\ \ non., if\ \ \eta_1 > S \\ sign., if\ \ \eta_2 > S > \eta_1 \\ adva., if\ \ \eta_3 > S > \eta_2 \\ cirr., if\ \ S > \eta_3 \end{cases} \quad \text{f} \quad \text{fi}$$

Comparison of the Chin-CHB score with the AAR, LS alone, APRI, and Hepascore was implemented with R software (www.R-project.org). The diagnostic accuracy for identifying the stage of hepatic fibrosis was assessed with the AUC, sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio with 95% confidence intervals (CIs).

## RESULTS

### Patient characteristics

Overall, 1,460 patients with HBV were enrolled and screened for eligibility. Among them, 260 patients were excluded because of decompensation (n = 71), undetectable HBV DNA (n = 64), absence of relevant serum markers (n = 46), unreliable LSM (n = 32), coexisting liver diseases (n = 14), withdrawal of informed consent (n = 14), inadequate liver tissue specimens for staging liver fibrosis (n = 10), and previous HBV therapy within 6 months (n = 9). Finally, 1,200 patients were included in the final analysis. According to the liver histological findings, 310 (25.8%) had cirrhosis, of whom 186 had normal ALT; 192 (16.0%) had F3 fibrosis; 456 (38.0%) had F2 fibrosis; and 242 (20.2%) had F0/1 fibrosis.

Among them, 800 patients were assigned to the training set and 400 patients to the validation set according to the regional representativeness and sample size. Most of the baseline variables were comparable between training and validation sets (Table 1). But the validation set had a higher proportion of patients with HBV DNA load $>5 \times 10^5$ IU/mL (60% vs 53%, $P = 0.018$) and higher quantitative HBsAg levels (3.13 vs 2.17, $P = 0.042$).

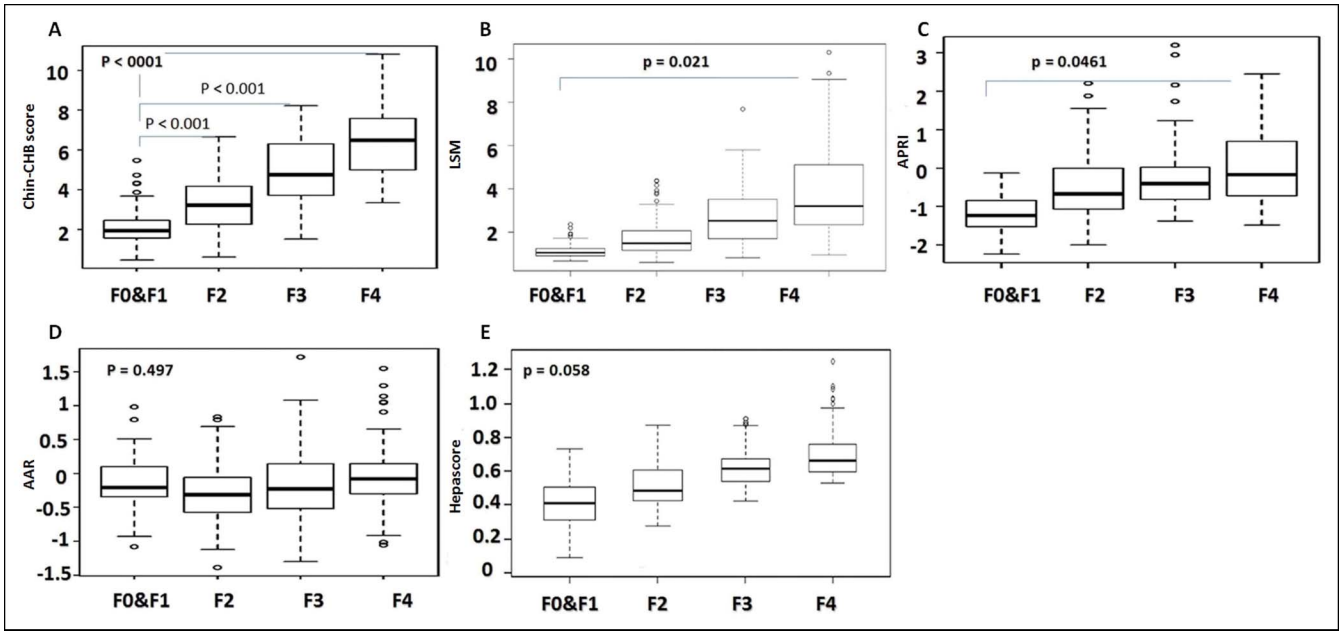


**Figure 3.** Difference in the Chin-CHB score (**a**), LSM (**b**), APRI (**c**), AAR (**d**), and Hepascore (**e**) among the 4 stages of liver fibrosis. AAR, alanine aminotransferase ratio; APRI, aspartate aminotransferase to platelet index; CHB, chronic hepatitis B; LSM, liver stiffness measurement.

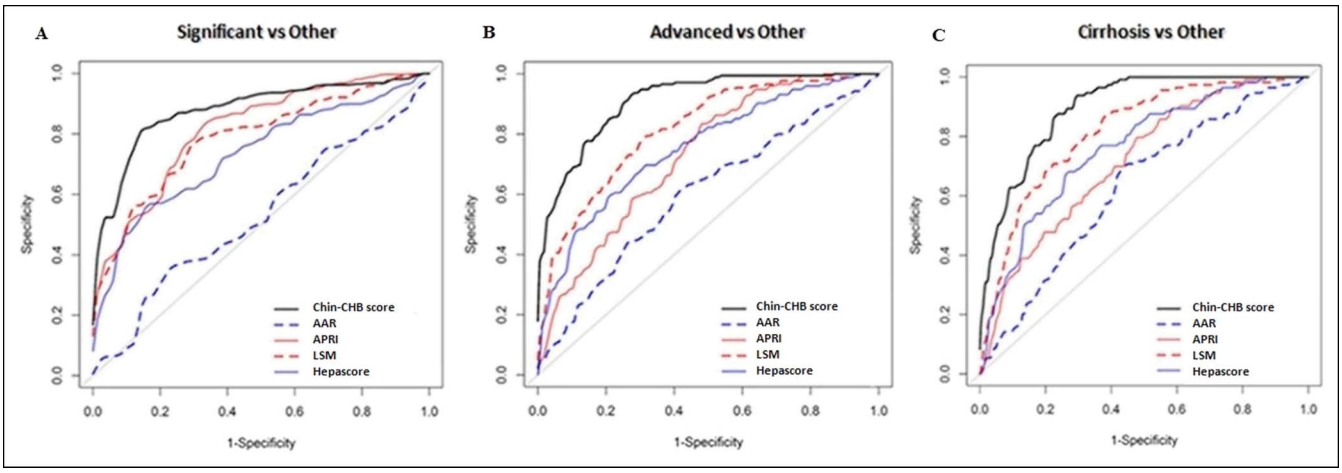


**Figure 4.** ROC analyses of the Chin-CHB score, LSM, APRI, AAR, and Hepascore for identifying the different stages of liver fibrosis. (**a**) Distinguishing significant fibrosis. (**b**) Distinguishing advanced fibrosis. (**c**) Distinguishing cirrhosis. AAR, alanine aminotransferase ratio; APRI, aspartate aminotransferase to platelet index; CHB, chronic hepatitis B; LSM, liver stiffness measurement; ROC, receiver operator characteristic curve.

### Predictors associated with stage of liver fibrosis

Except for the creatinine level, nearly all variables were significantly different among the 4 stages of liver fibrosis (see Figure 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A31). The top 5 significant predictors for distinguishing between significant fibrosis and mild fibrosis included drink, dSpleen, LSM, PLT, and HA (Figure 1a). The top 5 significant predictors for distinguishing between advanced fibrosis and nonadvanced fibrosis included age, LSM, PLT, dSpleen, and quantitative HBsAg level (Figure 1b). The top 5 significant predictors for distinguishing between cirrhosis and noncirrhosis included age, LSM, dSpleen, PLT, and AMG (Figure 1c). Based on the statistical results above, we calculated the mean selection frequency of all predictors and further ranked them. Consequently, LSM, PLT, age, HA, and dSpleen were the top 5 predictors associated with any stage of liver fibrosis (Figure 1d). Correlation heat map also suggested that the 5 predictors highly correlated with different stages of liver fibrosis, and the overlap was the minimal among each other (see Figure 2, Supplementary Digital Content 2, http://links.lww.com/CTG/A32).

### A novel noninvasive program for staging liver fibrosis

LSM, PLT, age, HA, and dSpleen were composed of a novel noninvasive program (i.e., Chin-CHB score). AUCs of the Chin-CHB score for diagnosing significant fibrosis, advanced fibrosis, and cirrhosis were 0.893 (95% CI: 0.77–0.92), 0.897 (95% CI: 0.85–0.95), and 0.909 (95% CI: 0.87–0.95), respectively. The accuracy of the Chin-CHB score for diagnosing significant fibrosis ($P = 0.882$, Figure 2a), advanced fibrosis ($P = 0.402$, Figure 2b), and cirrhosis ($P = 0.538$, Figure 2c) was similar between the training and validation sets. Finally, we developed an automated program, which can be available online.

### Comparison with other models for staging liver fibrosis

Chin-CHB score, LSM alone, and APRI score were significantly different among the stages of fibrosis ($P < 0.0001$, $P = 0.021$, and $P = 0.0461$, respectively), but not AAR or Hepascore ($P = 0.497$ and $P = 0.058$, respectively) (Figure 3a–e). The Chin-CHB score obtained in the validation cohort had higher accuracy than the APRI, AAR, LSM alone, and Hepascore for diagnosing significant fibrosis ($P < 0.001$, Figure 4a and Table 2), advanced fibrosis ($P < 0.001$, Figure 4b and Table 3), and cirrhosis ($P < 0.001$, Figure 4c and Table 4). Compared with other established models, the Chin-CHB score had the highest positive likelihood ratio and lowest negative likelihood ratio for diagnosing significant fibrosis (Table 2), advanced fibrosis (Table 3), and cirrhosis (Table 4).

## DISCUSSION

Liver biopsy is the golden standard for diagnosis and risk stratification of liver fibrosis, but is invasive, poorly compliant, and costly. Its wide application is neither realistic nor suitable for all patients in real-world clinical practice. Alternative approaches for noninvasive diagnosis of liver fibrosis are very attractive. They are primarily classified as individual serum biomarkers, scores combining demographic and clinical data and serum biomarkers, and imaging (see Figure 3, Supplementary Digital Content 3, http://links.lww.com/CTG/A33). Their specific properties lead to the respective advantages and disadvantages in diagnosing liver fibrosis. More recently, the researchers prefer to combine demographic and clinical data, serum biomarkers, and imaging to achieve better diagnostic performance.

This study had an intent to develop and validate a novel noninvasive program (i.e., Chin-CHB score) for accurately staging liver fibrosis in untreated patients with CHB. There are some advantages in conducting this study, as follows: (i) a large prospective database included 1,200 patients with CHB from multiple centers (800 patients for a training set and 400 patients for a validation set); (ii) the nature of the study population was relatively homogeneous (i.e., patients had HBV alone and were not receiving HBV therapy); (iii) a central pathological analysis was performed to unify the stage of liver fibrosis; (iv) except for cirrhosis as an observed end point, significant and advanced fibrosis were evaluated to prevent the "spectrum bias"; (v) the statistical results were similar between the training and validation sets, suggesting an excellent reproducibility of the new program; and (vi) 5 components produced to calculate the score are readily available for patients with CHB and often prescribed by hepatologists in

**Table 2. Comparison of the Chin-CHB score with other models for diagnosing significant fibrosis**

| | F0/F1 (n = 83) | F2–F4 (n = 317) | Cutoff value | AUC | Sensitivity | Specificity | PPV | NPV | LR+ | LR− |
|---|---|---|---|---|---|---|---|---|---|---|
| Chin-CHB score | | | | | | | | | | |
| ≤Cutoff (n) | 71 | 58 | 2.875 | 0.904 (0.86–0.92) | 0.82 (0.77–0.86) | 0.86 (0.78–0.93) | 0.96 (0.93–0.98) | 0.55 (0.46–0.64) | 5.65 (3.34–9.56) | 0.21 (0.17–0.27) |
| >Cutoff (n) | 12 | 259 | | | | | | | | |
| AAR | | | | | | | | | | |
| ≤Cutoff (n) | 53 | 209 | 1.103 | 0.47 (0.41–0.54) | 0.34 (0.32–0.43) | 0.64 (0.67–0.85) | 0.78 (0.75–0.88) | 0.20 (0.14–0.25) | 1.04 (0.83–1.52) | 1.03 (0.71–1.36) |
| >Cutoff (n) | 30 | 108 | | | | | | | | |
| APRI | | | | | | | | | | |
| ≤Cutoff (n) | 51 | 96 | 0.603 | 0.61 (0.77–0.87) | 0.70 (0.71–0.81) | 0.61 (0.64–0.83) | 0.87 (0.81–0.90) | 0.35 (0.30–0.39) | 1.87 (1.19–3.13) | 0.48 (0.04–0.91) |
| >Cutoff (n) | 32 | 221 | | | | | | | | |
| LSM | | | | | | | | | | |
| ≤Cutoff (n) | 55 | 98 | 4.342 | 0.79 (0.74–0.84) | 0.69 (0.70–0.80) | 0.66 (0.63–0.82) | 0.88 (0.82–0.93) | 0.35 (0.27–0.44) | 2.11 (1.53–2.86) | 0.49 (0.27–1.14) |
| >Cutoff (n) | 28 | 219 | | | | | | | | |
| Hepascore | | | | | | | | | | |
| ≤Cutoff (n) | 48 | 156 | 0.439 | 0.73 (0.68–0.79) | 0.51 (0.52–0.63) | 0.58 (0.75–0.91) | 0.82 (0.75–0.89) | 0.24 (0.17–0.41) | 1.39 (0.78–3.31) | 0.86 (0.44–1.61) |
| >Cutoff (n) | 35 | 161 | | | | | | | | |

AAR, aspartate aminotransferase/alanine aminotransferase ratio; APRI, AST to platelet index; AUC, area under the receiver operator characteristic curve; CHB, chronic hepatitis B; LR−, negative likelihood ratio; LR+, positive likelihood ratio; LSM, liver stiffness measurement with FibroScan; NPV, negative predictive value; PPV, positive predictive value.

**Table 3.** **Comparison of the Chin-CHB score with other models for diagnosing advanced fibrosis**

| | F0–F2 (n = 225) | F3–F4 (n = 175) | Cutoff value | AUC (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | LR+ (95% CI) | LR− (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Chin-CHB score | | | | | | | | | | |
| ≤Cutoff (n) | 192 | 24 | 4.06 | 0.91 (0.88–0.94) | 0.86 (0.80–0.90) | 0.85 (0.74–0.88) | 0.82 (0.7–0.89) | 0.89 (0.83–0.92) | 5.73 (4.19–7.43) | 0.16 (0.08–0.27) |
| >Cutoff (n) | 33 | 151 | | | | | | | | |
| AAR | | | | | | | | | | |
| ≤Cutoff (n) | 132 | 67 | 0.984 | 0.6 (0.54–0.66) | 0.62 (0.55–0.69) | 0.59 (0.52–0.65) | 0.54 (0.47–0.61) | 0.66 (0.6–0.73) | 1.49 (1.23–1.81) | 0.65 (0.52–0.81) |
| >Cutoff (n) | 93 | 108 | | | | | | | | |
| APRI | | | | | | | | | | |
| ≤Cutoff (n) | 134 | 49 | 1.223 | 0.73 (0.68–0.78) | 0.72 (0.65–0.79) | 0.60 (0.53–0.66) | 0.58 (0.51–0.65) | 0.73 (0.67–0.8) | 1.78 (1.48–2.14) | 0.47 (0.36–0.61) |
| >Cutoff (n) | 91 | 126 | | | | | | | | |
| LSM | | | | | | | | | | |
| ≤Cutoff (n) | 166 | 47 | 7.273 | 0.82 (0.77–0.86) | 0.73 (0.67–0.8) | 0.74 (0.68–0.8) | 0.68 (0.62–0.75) | 0.78 (0.72–0.84) | 2.89 (2.2–3.53) | 0.36 (0.28–0.47) |
| >Cutoff (n) | 59 | 128 | | | | | | | | |
| Hepascore | | | | | | | | | | |
| <Cutoff (n) | 155 | 53 | 0.522 | 0.75 (0.71–0.8) | 0.7 (0.63–0.77) | 0.69 (0.63–0.75) | 0.64 (0.57–0.7) | 0.75 (0.69–0.8) | 2.24 (1.8–2.79) | 0.44 (0.35–0.56) |
| >Cutoff (n) | 70 | 122 | | | | | | | | |

AAR, aspartate aminotransferase/alanine aminotransferase ratio; APRI, AST to platelet index; AUC, area under the receiver operator characteristic curve; CHB, chronic hepatitis B; CI, confidence interval; LR−, negative likelihood ratio; LR+, positive likelihood ratio; LSM, liver stiffness measurement with FibroScan; NPV, negative predictive value; PPV, positive predictive value.

**Table 4. Comparison of the Chin-CHB score with other models for diagnosing cirrhosis**

| | F0–F3 (n = 287) | F4 (n = 113) | Cutoff value | AUC | Sensitivity | Specificity | PPV | NPV | LR+ | LR− |
|---|---|---|---|---|---|---|---|---|---|---|
| Chin-CHB score | | | | | | | | | | |
| ≤Cutoff (n) | 268 | 10 | 4.402 | 0.90 (0.83–0.95) | 0.91 (0.86–0.94) | 0.93 (0.88–0.97) | 0.84 (0.80–0.92) | 0.96 (0.91–0.97) | 13.19 (9.1–18.78) | 0.11 (0.06–0.27) |
| >Cutoff (n) | 19 | 103 | | | | | | | | |
| AAR | | | | | | | | | | |
| ≤Cutoff (n) | 164 | 35 | 1.104 | 0.63 (0.57–0.69) | 0.69 (0.61–0.78) | 0.57 (0.51–0.63) | 0.39 (0.32–0.46) | 0.82 (0.77–0.88) | 1.61 (1.34–1.93) | 0.54 (0.4–0.73) |
| >Cutoff (n) | 123 | 78 | | | | | | | | |
| APRI | | | | | | | | | | |
| ≤Cutoff (n) | 192 | 43 | 1.675 | 0.72 (0.66–0.77) | 0.62 (0.53–0.71) | 0.67 (0.61–0.72) | 0.42 (0.35–0.5) | 0.82 (0.77–0.87) | 1.87 (1.5–2.33) | 0.57 (0.44–0.73) |
| >Cutoff (n) | 95 | 70 | | | | | | | | |
| LSM | | | | | | | | | | |
| ≤Cutoff (n) | 247 | 20 | 14.676 | 0.82 (0.77–0.86) | 0.82 (0.72–0.88) | 0.86 (0.73–0.93) | 0.70 (0.61–0.77) | 0.91 (0.83–0.96) | 5.63 (2.52–7.13) | 0.23 (0.15–0.51) |
| >Cutoff (n) | 40 | 93 | | | | | | | | |
| Hepascore | | | | | | | | | | |
| ≤Cutoff (n) | 211 | 36 | 0.697 | 0.76 (0.71–0.81) | 0.68 (0.6–0.77) | 0.74 (0.68–0.79) | 0.50 (0.42–0.58) | 0.85 (0.81–0.9) | 2.57 (2.04–3.24) | 0.43 (0.33–0.57) |
| >Cutoff (n) | 76 | 77 | | | | | | | | |

AAR, aspartate aminotransferase/alanine aminotransferase ratio; APRI, AST to platelet index; AUC, area under the receiver operator characteristic curve; CHB, chronic hepatitis B; LR−, negative likelihood ratio; LR+, positive likelihood ratio; LSM, liver stiffness measurement with FibroScan; NPV, negative predictive value; PPV, positive predictive value.

regular clinical practice. Our study found that the new program had a high diagnostic performance for different stages of liver fibrosis and that its diagnostic performance was better than other established models and methods (i.e., APRI, AAR, LSM alone, and Hepascore).

A potential explanation for better diagnostic performance of the new program might be an effective combination of 5 variables from various directions. First, LSM, an elastic variable, is rapid and easy to perform in clinical practice (14). Notably, LSM has the greatest power among these components for the new program. However, the diagnostic performance of LSM is readily influenced by the nature of selected population. Among patients with intermediate stages of fibrosis or hepatic inflammation, the diagnostic performance of LSM may be reduced. Second, PLT and dSpleen are 2 important markers for noninvasive assessment of esophageal varices (19–21), which are associated with an increased portal pressure. Baveno VI consensus also recommends the usefulness of PLT and dSpleen for ruling in clinically significant portal hypertension (22). Indeed, both esophageal varices and clinically significant portal hypertension can reflect the stages of severe fibrosis and cirrhosis in chronic liver diseases. Third, age, an important demographic variable, is positively associated with progression of chronic liver diseases. In China, where HBV is endemic (23), mother-to-child transmission has been recognized as one of the most common approaches of CHB (24). It is reasonable that the severity and progression of liver injury since HBV infection by perinatal transmission correlate with the age of untreated individuals with CHB. Fourth, HA, a serum marker, is primarily synthesized in the liver (25). Degradation of HA is gradually impaired with progression of liver diseases. Earlier studies suggested the role of HA for the assessment of liver fibrosis (26). Taken together, the 5 variables from 4 major dimensions, including one elastic marker for liver fibrosis, 2 markers for portal hypertension, one demographic marker, and one serum marker for liver fibrosis, were integrated into a program to further improve the diagnostic performance.

This study had several limitations. First, this is a cross-sectional study without any longitudinal follow-up data to assess the significance of this new program for the progression or regression of liver fibrosis in patients with CHB. Second, other liver elasticity-based imaging techniques, such as point shear-wave elastography, 2D shear-wave elastography, and magnetic resonance elastography, are not compared in this study. Third, other serum biomarkers, such as Fibrometer, Lok index, and Hui score, are not compared in this study. Fourth, external validation in other racial populations is lacking. Fifth, intercenter variations in the laboratory testing cannot be avoided among centers, despite the training was performed in collecting the blood samples, selecting the same assays, and measuring the LSM before starting this trial.

In conclusion, we developed and validated a novel noninvasive program, which was composed of LSM, PLT, age, HA, and dSpleen, for assessing the stage of liver fibrosis in Chinese untreated patients with CHB (see Figure 4, Supplementary Digital Content 4, http://links.lww.com/CTG/A34). Generally, this novel program is not only cheap and readily available but also more accurate than several established models or methods. Certainly, external validation should be warranted before its application in clinical practice.

### CONFLICTS OF INTEREST

**Guarantor of the article:** Yongping Yang, MD, PhD.

**Specific author contributions:** Yan Chen, MD, PhD, Yongji Wang, PhD, Yongping Chen, MD, Zujiang Yu, MD, PhD, Xiaoling Chi, MD, Ke-Qin Hu, MD, and Qin Li, MD, contributed equally to this work. Y.C. wrote the protocol, collected the data, performed the statistical analysis, interpreted the data, and drafted the manuscript. Y.W. and K.-Q.H. interpreted the data and revised the manuscript. Y.C., Z.Y., X.C., Q.L., L.T., D.X., Q.S., C.L., L.C., X.H., J.W., H.L., W.L., W.C., Z.D., X.W., Z.L., H.X., D.C., W.B., C.Z., G.X., X.Q., J.C., L.Z., H.S., M.D. collected the data, interpreted the data, and gave critical comments. X.Q. interpreted the data and gave critical comments. Z.Z. performed the statistical analysis, interpreted the data, and drafted the manuscript. X.Q. interpreted the data, gave critical comments, and drafted and revised the manuscript. Y.Y. conceived the work, wrote the protocol, collected the data, performed the statistical analysis, interpreted the data, had the access to all data, and drafted and revised the manuscript. All authors have made an intellectual contribution to the manuscript and approved the submission.

**Funding support:** This work was supported in part by the grant from the National Major Science and Technology Special Project of China (2013ZX10005002).

**Potential Competing interests:** None.

### ACKNOWLEDGEMENTS

The authors thank all the individuals who participated in this study.

## Study Highlights

### WHAT IS KNOWN

- ✓ Early and accurate diagnosis of liver fibrosis/cirrhosis is important.
- ✓ Currently, liver biopsy remains the gold standard approach for staging liver fibrosis, but has some limitations.
- ✓ Several noninvasive serum models have been developed, but their diagnostic performance is unsatisfactory.
- ✓ LSM measurement seems to have an improved diagnostic performance, but is questioned in middle stages of fibrosis.

### WHAT IS NEW HERE

- ✓ A novel noninvasive program, named as the Chin-CHB score, is developed and validated in this cross-sectional study involving 1,200 patients with CHB from 14 Chinese centers. It is composed of 5 regular variables (i.e., LSM, platelet, age, HA, and dSpleen).
- ✓ The Chin-CHB score has better diagnostic performance for any stage of liver fibrosis than several established models and methods, including a ratio of AST to ALT, AST to platelet index, Hepascore, and LSM.

### TRANSLATIONAL IMPACT

- ✓ In future, the physicians may use the Chin-CHB score to easily and accurately assess the stage of liver fibrosis and avoid the invasiveness of liver biopsy.

### REFERENCES

1. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384:2053–63.

2. Tang LSY, Covert E, Wilson E, et al. Chronic hepatitis B infection: A review. JAMA 2018;319:1802–13.
3. Iloeje UH, Yang HI, Jen CL, et al Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol 2007;5:921–31.
4. Rockey DC, Caldwell SH, Goodman ZD, et al. Liver biopsy. Hepatology 2009;49:1017–44.
5. McGill DB, Rakela J, Zinsmeister AR, et al. A 21-year experience with major hemorrhage after percutaneous liver biopsy. Gastroenterology 1990;99:1396–400.
6. Oberti F, Valsesia E, Pilette C, et al. Noninvasive diagnosis of hepatic fibrosis or cirrhosis. Gastroenterology 1997;113:1609–16.
7. Lee S, Kim do Y. Non-invasive diagnosis of hepatitis B virus-related cirrhosis. World J Gastroenterol 2014;20:445–59.
8. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: A prospective study. Lancet 2001;357:1069–75.
9. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis: Relationship to cirrhosis. Gastroenterology 1988;95:734–9.
10. 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.
11. Adams LA, Bulsara M, Rossi E, et al. Hepascore: An accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005; 51:1867–73.
12. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: A systemic review and meta-analysis. Hepatology 2015;61:292–302.
13. Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. Hepatology 2006;43:S113–20.
14. EASL-ALEH Clinical Practice Guidelines. Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63:237–64.
15. Stefanescu H, Procopet B. Noninvasive assessment of portal hypertension in cirrhosis: Liver stiffness and beyond. World J Gastroenterol 2014;20: 16811–9.
16. Sebastiani G, Castera L, Halfon P, et al. The impact of liver disease aetiology and the stages of hepatic fibrosis on the performance of non-invasive fibrosis biomarkers: An international study of 2411 cases. Aliment Pharmacol Ther 2011;34:1202–16.
17. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20:15–20.
18. McCullagh P. Regression models for ordinal data. J R Stat Soc Ser B Stat Methodol 1980;42:109–42.
19. Giannini E, Botta F, Borro P, et al. Platelet count/spleen diameter ratio: Proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. Gut 2003; 52:1200–5.
20. Giannini EG, Zaman A, Kreil A, et al. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: Results of a multicenter, prospective, validation study. Am J Gastroenterol 2006; 101:2511–9.
21. Chen R, Deng H, Ding X, et al. Platelet count to spleen diameter ratio for the diagnosis of gastroesophageal varices in liver cirrhosis: A systematic review and meta-analysis. Gastroenterol Res Pract 2017;2017:7407506.
22. de Franchis R. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol 2015;63:743–52.
23. Wang FS, Fan JG, Zhang Z, et al. The global burden of liver disease: The major impact of China. Hepatology 2014;60:2099–108.
24. 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.
25. Gudowska M, Cylwik B, Chrostek L. The role of serum hyaluronic acid determination in the diagnosis of liver fibrosis. Acta Biochim Pol 2017;64: 451–7.
26. Engstrom-Laurent A, Loof L, Nyberg A, et al. Increased serum levels of hyaluronate in liver disease. Hepatology 1985;5:638–42.