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Routine indexes for cirrhosis and significant fibrosis detection in patients with compensated chronic hepatitis B

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ABSTRACT

Background and aim: Fibrosis index based on the four factors (FIB-4) and aspartate aminotransferase to platelet ratio index (APRI) were not well validated in patients with chronic hepatitis B (CHB). The aim of this study was to validate the performances of these indexes and construct novel indexes for liver fibrosis assessment.
Methods: A total of 1438 consecutive antivirus treatment-naïve patients with CHB were analysed, and two novel indexes (named HeBCI and HeBFI) were derived for cirrhosis and significant fibrosis detection.
Results: For cirrhosis, the area under receiver operating characteristic curves (AUROCs) were 0.841 for HeBCI, 0.708 for FIB-4 and 0.623 for APRI in the model set, and 0.779, 0.690, 0.595 in the validation set. For significant fibrosis, the AUROCs were 0.781 for HeBFI, 0.693 for APRI and 0.641 for FIB-4 in the model set, and 0.776, 0.729, 0.641 in the validation set. HeBCI determined 750 (52.2%) patients as having cirrhosis or non-cirrhosis with an accuracy of 86%. HeBFI detected 673 (46.8%) patients with or without significant fibrosis with an accuracy of 76.6%.
Conclusions: As economical and convenient indexes, HeBCI and HeBFI are suitable to serve as outpatient tools for detecting significant fibrosis and cirrhosis to reduce the need of liver biopsy significantly in resource-limited settings.

1. Introduction

Hepatitis B virus (HBV) affects 350 million people in the world and almost one million individuals die from HBV-related liver diseases such as cirrhosis, hepatocellular carcinoma and acute on chronic liver failure every year [1]. The cumulative incidences of cirrhosis in a span of 5 years were 8.0% and 13.0% for hepatitis B e antigen (HBeAg) positive and HBeAg negative chronic hepatitis B (CHB) in patients, respectively [2]. The clinical significance of fibrosis assessment in patients with CHB is to predict the clinical prognosis and screen the high-risk patients who need active antiviral therapy to prevent cirrhosis development [3,4].

Liver biopsy is the gold standard for assessment of fibrosis but is limited by its invasiveness and susceptibility to sampling error [5,6]. Transient elastography has good diagnostic value in detecting fibrosis but may not be available in source-limited settings. More important, failures to obtain enough measurements have been reported in 3% of cases, and unreliable results have been reported in 16% of cases, mostly due to obesity, ascites and limited operator experience [7]. In cases of acute hepatitis, extrahepatic cholestasis and congestion might result in false positive diagnoses and reduce the diagnostic accuracy of fibrosis assessment [8,9]. From the perspective of cost-effectiveness and clinical application, an ideal diagnostic index for fibrosis assessment should be characterised by the following advantages: simple, non-invasive, economical, convenient and easily repeatable.

Aspartate aminotransferase-to-platelet ratio index (APRI) and fibrosis index based on the four factors (FIB-4) have been constructed to evaluate hepatic fibrosis [10,11]. APRI and FIB-4, which are more readily available, economical and easy to perform in

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an outpatient setting, were recommended for hepatic fibrosis in resource-limited settings by the World Health Organization in 2015 [12]. However, the diagnostic performances and clinical utility of these indexes for hepatic fibrosis assessment in CHB patients were uncertain. First, these indexes lacked large external studies to validate their diagnostic accuracy in different populations. Second, the range of hepatic fibrosis stages might have a major influence on variability in assessing the diagnostic accuracy of non-invasive indexes [13]. Thus, the influence of the numerous hepatic fibrosis stages in various studies should be taken into account when evaluating the diagnostic accuracy of non-invasive indexes.

Therefore, we performed this retrospective study to construct novel diagnostic indexes for hepatic fibrosis in CHB patients as well as to compare their diagnostic accuracy with APRI and FIB-4.

2. Patients and methods

2.1. Patients

This retrospective study included 2152 consecutive patients with CHB between January 2008 and November 2014. The patients were enrolled based on the following criteria: chronic HBV infection defined as hepatitis B surface antigen positivity for more than 6 months. The exclusion criteria were defined as follows: inadequate biopsy sample defined as specimen length <15 mm (n = 76); co-infection with hepatitis C virus, hepatitis D virus or human immunodeficiency virus (n = 51) and other liver disease (n = 10), including liver cancer, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis and Wilson's disease. Patients with missing data (n = 207) of important study variables (including α-fetoprotein, γ-glutamyl transferase, total cholesterol, aspartate aminotransferase (AST), age, platelet and albumin) and who underwent antivirus treatment (n = 370) were also excluded from analysis (Supplement Figure). A total of 1438 consecutive antivirus treatment-naïve patients were recruited in the final analysis.

All information collections and clinical investigations were performed according to the principles of the Declaration of Helsinki. The study was approved by the ethics committee of Shunde Hospital, Southern Medical University.

2.2. Liver biopsy

Liver biopsies were performed by two experienced physicians, using a 16-gauge needle. Only the liver sample with specimen length ≥15 mm was recruited in the present study. The specimens were fixed, paraffin-embedded and stained with haematoxylin and eosin. Histological grading of necroinflammation activity (A0–A4) and the liver fibrosis stage (S0–S4) were scored according to Scheuer classification by one experienced pathologist blinded to the clinical data. In the present study, significant fibrosis (SF) was defined as fibrosis stage ≥ S2, and cirrhosis was defined as fibrosis stage S4.

2.3. Serum markers and non-invasive models

All patients underwent complete biochemical workups, complete blood count, α-fetoprotein (AFP, ng/ml), HBeAg and liver biopsy within 2 days. Biochemical tests were performed by commercial assays in our hospital laboratory for alanine aminotransferase (ALT, U/L), AST (U/L), γ-glutamyl transferase (GGT, U/L), bilirubin (mmol/L), albumin (g/L), globulin (g/L), creatinine (μmol/L), fasting plasma glucose (mmol/L), total cholesterol (TC, mmol/L), triglyceride (mmol/L), high-density lipoprotein (mmol/L) and low-density lipoprotein (mmol/L).

The formulas of APRI and FIB-4 were calculated as described in the original articles [10,11]. APRI = AST(ULN)/platelet (10^9/L) × 100; FIB-4 = age (year) × AST(U/L)/[PLT (10^9/L) × ALT (U/L)$^{1/2}$].

2.4. Standardisation of area under receiver operating characteristic curve according to the prevalence of fibrosis stages

It has been reported that the prevalence of liver fibrosis stages may be a major factor of variability in assessing the diagnostic accuracy of non-invasive models [13]. The area operating characteristic curve (AUROC) was adjusted according to the prevalence of fibrosis stages by using the difference between advanced and non-advanced fibrosis (DANA) [13]. DANA was calculated according to the following formula: for SF, DANA = [(prevalence S2 × 2 + prevalence S3 × 3 + prevalence S4 × 4)/(prevalence S2 + prevalence S3 + prevalence S4)] − [prevalence S1/(prevalence S0 + prevalence S1)]; for cirrhosis, DANA = [(prevalence S4 × 4)/(prevalence S4)] − [prevalence S1 + prevalence S2 × 2 + prevalence S3 × 3/(prevalence S0 + prevalence S1 + prevalence S2 + prevalence S3)]. The adjusted AUROCs (Adj-AUROC) were calculated according to the following formula: Adj-AUROC = AUROC + 0.1056 × (2.5 − DANA).

2.5. Statistical analysis

Continuous data were expressed as mean ± standard deviation or median (25th–75th percentiles), depending on the normality of the data. Continuous variables were compared by *t*-test or Mann–Whitney U test as appropriate. Categorical variables were compared by chi-squared test or Fisher's exact test as appropriate. Several continuous variables were translated to logarithmic scale in cases of logarithmic conversion to improve its contribution to the dependent variable, according to R square in univariate logistic regression. All variables significantly associated with fibrosis in univariate logistic regression analysis were included in forward stepwise multivariate logistic regression analysis to derive diagnostic indexes for SF and cirrhosis. The AUROCs were calculated to evaluate the performance of diagnostic indexes and compared by DeLong's test. Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL). All statistical tests were two-sided. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of subjects in model set and validation set

A total of 1438 consecutive antivirus-naïve patients with CHB were finally recruited in the present study. Table 1 shows the clinical characteristics of patients included in the final analysis. To explore the diagnostic performances in different study populations with different baseline characteristics, patients were divided into model set (n = 813) and validation set (n = 625), according to the admission time.

3.2. Diagnostic index for cirrhosis detection

Variables, including AFP, GGT, age, platelet and albumin, were included in the hepatitis B cirrhosis index (HeBCI) (Table 2).

$$\begin{aligned}\text{HeBCI} = {} & \exp(8.866 + 0.029 \times \text{age} - 0.054 \times \text{albumin} + 0.368 \\ & \times \log_e \text{GGT} + 0.478 \times \log_e \text{AFP} - 2.246 \times \log_e \text{platelet})/\{1 \\ & + \exp(8.866 + 0.029 \times \text{age} - 0.054 \times \text{albumin} + 0.368 \times \log_e \\ & \text{GGT} + 0.478 \times \log_e \text{AFP} - 2.246 \times \log_e \text{platelet})\}\end{aligned}$$

Table 1
Clinical characteristics of patients included in final analysis.

	Overall n = 1438	Model set n = 813	Validation set n = 625	Test value	*P*
Age (year)	33.0 ± 9.6	32.0 ± 9.6	34.2 ± 9.5	4.388	0.001
Male (n, %)	1115(77.5)	631(77.6)	484(77.4)	0.01	0.938
Alanine aminotransferase (U/L)	82(43,169)	84(44,167)	82(42,177)	0.120	0.905
Aspartate aminotransferase (U/L)	57(39,97)	57(41,97)	56(37,97)	0.989	0.323
γ-Glutamyl transferase (U/L)	54(29,105)	52(30,99)	57(29,111)	0.734	0.463
Albumin (g/L)	44.0 ± 4.9	44.4 ± 5.0	43.5 ± 4.8	3.674	0.001
Bilirubin (μmol/L)	15.8(12.0, 20.8)	16.1(12.5, 21.6)	15.1(11.3, 20.0)	3.026	0.002
Creatinine (μmol/L)	79.5 ± 23.1	82.8 ± 26.3	75.2 ± 17.1	6.318	0.001
Fasting plasma glucose (mmol/L)	4.7 ± 1.2	4.5 ± 1.2	4.9 ± 1.2	5.228	0.001
Total cholesterol (mmol/L)	4.4 ± 1.0	4.5 ± 1.0	4.4 ± 1.0	0.627	0.531
Triglyceride (mmol/L)	1.2 ± 0.6	1.16 ± 0.6	1.2 ± 0.7	0.268	0.789
HDL (mmol/L)	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	4.414	0.001
LDL (mmol/L)	2.4 ± 0.8	2.4 ± 0.8	2.3 ± 0.7	3.571	0.001
White blood cell (10^9/L)	5.8 ± 1.6	5.9 ± 1.6	5.7 ± 1.5	2.304	0.021
Hemoglobin (g/L)	143.5 ± 16.5	143.2 ± 16.6	143.8 ± 16.3	0.748	0.454
Platelet (10^9/L)	186.2 ± 54.4	186.2 ± 55.2	186.1 ± 53.4	0.027	0.978
HBV DNA ($\log_{10}$ IU/ml)	5.1 ± 2.4	5.1 ± 2.4	5.1 ± 2.4	0.198	0.843
HBeAg+ (n, %)	950(66.1)	547(67.3)	403(64.5)	2.39	0.122
α-Fetoprotein (ng/ml)	6.1(3.2,11.6)	6.4(3.5,12.7)	5.6(2.8,10.6)	3.612	0.001
Inflammation activity				7.3	0.007
A0–1 (n, %)	69(4.8)	27(3.3)	42(6.7)		
A2 (n, %)	642(44.6)	359(44.2)	283(45.3)		
A3 (n, %)	526(36.6)	297(36.5)	229(36.6)		
A4 (n, %)	201(14.0)	130(16.0)	71(11.4)		
Fibrosis stage				0.32	0.569
S1 (n, %)	224(15.6)	148(18.2)	76(12.2)		
S2 (n, %)	522(36.3)	263(32.3)	259(41.1)		
S3 (n, %)	407(28.3)	220(27.1)	187(29.9)		
S4 (n, %)	285(19.8)	182(22.4)	103(16.5)		
FIB-4	1.2(0.8,1.9)	1.1(0.8,1.9)	1.2(0.7,2.0)	1.098	0.272
APRI	0.8(0.5,1.4)	0.8(0.5,1.4)	0.8(0.50,1.5)	0.494	0.621

Continuous variables were expressed as mean ± standard deviation or median (25th–75th percentiles) as appropriate.
HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; FIB-4: fibrosis index based on the four factors; APRI: aspartate aminotransferase to platelet ratio index.

Table 2
Univariate and multivariate analysis for variables included in the index predicting cirrhosis.

	Non-cirrhosis (n = 631)	Cirrhosis (n = 182)	*P*	Univariate analysis		Multivariate analysis		
				OR	*P*	Coefficient	*P*	OR (95% CI)
Male (n, %)	475(75.3)	156(85.7)	0.003	1.971(1.253–3.099)	0.003			
Age (year)	30.5 ± 8.5	37.2 ± 11.2	0.001	1.071(1.052–1.090)	0.001	0.036	0.001	1.037(1.015–1.059)
ALT (U/L)	86.0(47.0,163.0)	70.0(40.0,172.0)	0.329	1.000(0.999–1.002)	0.624			
AST (U/L)	58.0(41.0,94.0)	55.0(43.0,108.0)	0.386	1.002(1.000–1.004)	0.092			
GGT (U/L)	46.0(27.0,86.0)	81.0(48.0,141.0)	0.001	1.005(1.003–1.006)	0.001			
GGT (loge U/L)	3.9 ± 0.8	4.4 ± 0.8	0.001	2.218(1.787–2.753)	0.001	0.399	0.004	1.490(1.136–1.955)
Albumin (g/L)	45.2 ± 4.7	41.7 ± 5.1	0.001	0.836(0.801–0.873)	0.001	−0.078	0.002	0.925(0.880–0.971)
TBIL (μmol/L)	17.9 ± 10.1	21.4 ± 12.1	0.001	1.027(1.012–1.042)	0.001			
Creatinine (μmol/L)	81.5 ± 17.6	87.6 ± 44.8	0.006	1.010(1.001–1.019)	0.029			
FPG (mmol/L)	4.5 ± 1.1	4.6 ± 1.5	0.231	1.082(0.950–1.233)	0.236			
TC (mmol/L)	4.5 ± 1.0	4.3 ± 1.0	0.002	0.764(0.645–0.907)	0.002			
Triglyceride (mmol/L)	1.1 ± 0.6	1.2 ± 0.6	0.392	1.130(0.853–1.497)	0.393			
HDL (mmol/L)	1.3 ± 0.4	1.2 ± 0.4	0.001	0.409(0.264–0.633)	0.001			
LDL (mmol/L)	2.5 ± 0.8	2.3 ± 0.8	0.026	0.785(0.634–0.972)	0.026			
WBC (10^9/L)	5.9 ± 1.5	5.7 ± 1.9	0.169	0.917(0.823–1.021)	0.114			
hemoglobin (g/L)	144.5 ± 16.3	138.4 ± 17.1	0.001	0.979(0.969–0.989)	0.001			
Platelet (10^9/L)	194.7 ± 51.5	156.8 ± 57.7	0.001	0.985(0.982–0.988)	0.001			
Platelet (loge 10^9/L)	5.2 ± 0.3	5.0 ± 0.4	0.001	0.079(0.043–0.143)	0.001	−2.179	0.001	0.113(0.058–0.220)
AFP (ng/ml)	5.8(3.2,9.8)	13.1(6.4,40.0)	0.001	1.003(1.002–1.005)	0.001			
AFP (loge ng/ml)	1.8 ± 1.0	2.9 ± 1.5	0.001	2.012(1.727–2.344)	0.001	0.406	0.001	1.500(1.250–1.800)
HBV DNA (lg IU/ml)	5.2 ± 2.4	4.8 ± 2.3	0.033	0.931(0.871–0.994)	0.034			
Constant						9.036	0.001	

Continuous variables were expressed as mean ± standard deviation or median (25th–75th percentiles) as appropriate.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transferase; TBIL: total bilirubin; FPG: fasting plasma glucose; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; WBC: white blood cell; AFP: α-fetoprotein.

Fig. 1A and B shows the AUROCs of cirrhosis detection. In the model set, the AUROC of HeBCI (0.841, 95% confidence interval 0.808–0.873) was superior to that of FIB-4 (0.708, 0.664–0.751) and APRI (0.623, 0.578–0.667) ($P < 0.001$), with the adj-AUROCs 0.906, 0.773 and 0.688 in the same order. In the validation set, the AUROC of HeBCI (0.779, 0.729–0.828) was also superior to that of FIB-4 (0.690, 0.635–0.745) and APRI (0.595, 0.536–0.654) ($P < 0.001$), with the adj-AUROCs 0.853, 0.765 and 0.670 in the same order.

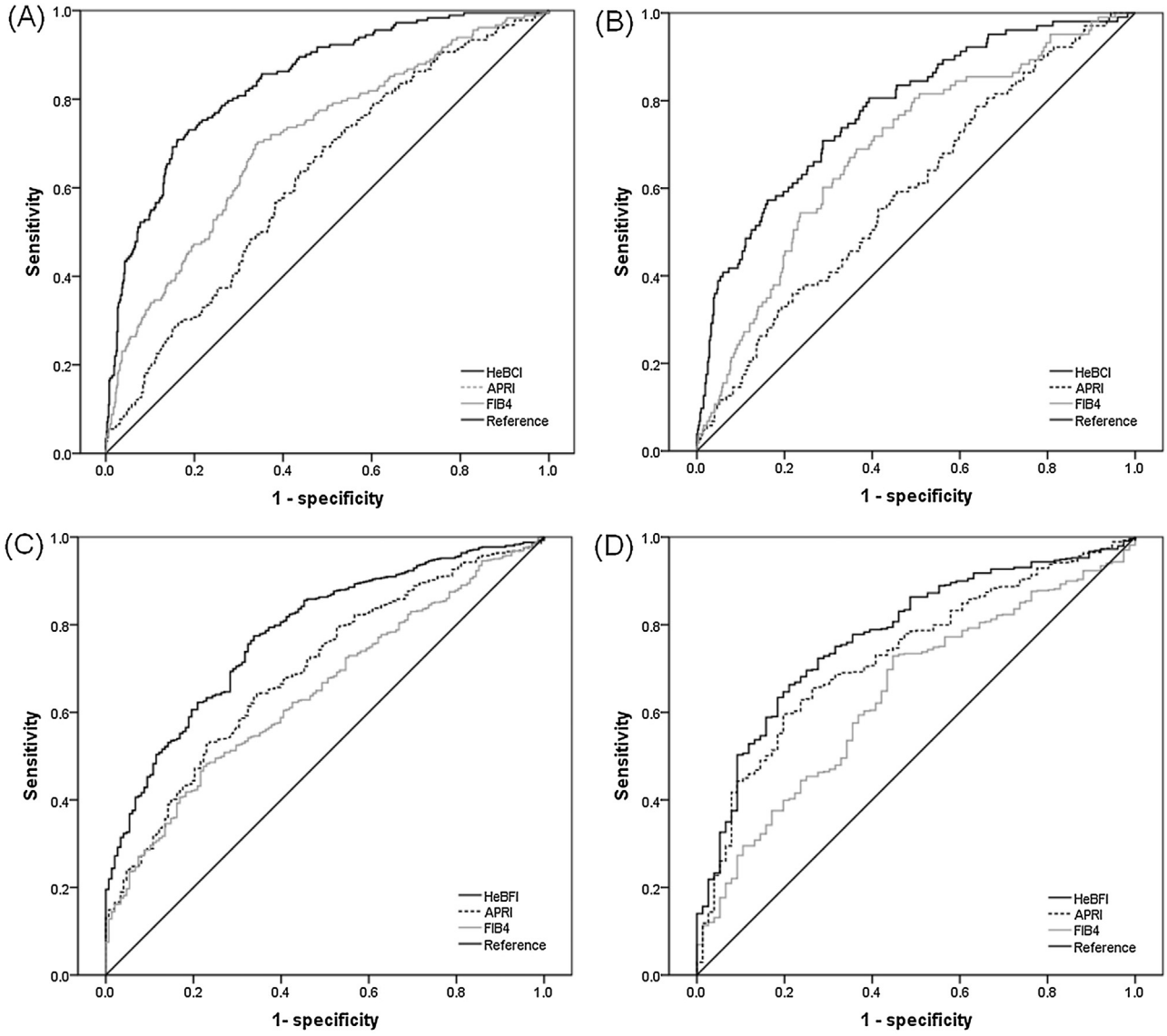

Fig. 1. Receiver operating characteristics curves for routine diagnostic indexes. Cirrhosis detection in model set (A) and validation set (B); significant fibrosis detection in model set (C) and validation set (D). **HeBCI:** hepatitis B cirrhosis index; **HeBFI:** hepatitis B significant fibrosis index; **FIB-4:** fibrosis index based on the four factors; **APRI:** aspartate aminotransferase-to-platelet ratio index.

3.3. Diagnostic index for significant fibrosis detection

Variables, including TC, AST, GGT, AFP, platelet and albumin, were included in the hepatitis B significant fibrosis index (HeBFI) (Table 3).

$$\text{HeBFI} = \exp(8.862 + 0.321 \times \log_e \text{GGT} + 0.012 \times \text{AST-0.069} \times \text{albumin-0.261} \times \text{TC} + 0.469 \times \log_e \text{AFP-1.119} \times \log_e \text{platelet}) / \{1 + \exp(8.862 + 0.321 \times \log_e \text{GGT} + 0.012 \times \text{AST-0.069} \times \text{albumin-0.261} \times \text{TC} + 0.469 \times \log_e \text{AFP-1.119} \times \log_e \text{platelet})\}$$

Fig. 1C and D shows the AUROCs of SF detection. The AUROC of HeBFI (0.781, 0.724–0.828) was superior to that of APRI (0.693, 0.648–0.737) and FIB-4 (0.641, 0.601–0.691) in the model set ($P < 0.001$), with the adj-AUROCs 0.847, 0.759 and 0.707 in the same order. In the validation set, the AUROC of HeBFI (0.776, 0.724–0.828) was superior to that of APRI (0.729, 0.674–0.785) and FIB-4 (0.641, 0.578–0.703) ($P < 0.001$), with the adj-AUROCs 0.859, 0.812 and 0.724 in the same order.

3.4. Clinical application of routine indexes for cirrhosis and significant liver fibrosis detection

Table 4 shows the diagnostic evaluation of the routine indexes for cirrhosis and SF detection.

The higher cut off 0.40 of HeBCI was characterised by a positive likelihood ratio (PLR) of 7.9, specificity 94.3% and positive predictive value (PPV) 69.5% in the model set; and PLR 5.22, specificity 92.0% and PPV 50.6% in the validating set. The lower cut off 0.09 of HeBCI excluded cirrhosis diagnosis with negative likelihood ratio (NLR) of 0.16, sensitivity 92.3% and negative predictive value (NPV) 95.5% in the model set; and NLR 0.25, sensitivity 89.3% and NPV 95.3% in the validating set. These cut-offs included cirrhosis diagnosis with a PLR higher than 5 and excluded a cirrhosis diagnosis with NLR of nearly 0.2, which were at least characterised by moderate statistical evidence [14]. Among the total of 1438 patients with overall accuracy of 86%, 205 (14.3%) patients with HeBCI > 0.40

Table 3
Univariate and multivariate analysis for variables included in the index predicting significant fibrosis.

	Non-SF (n = 148)	SF (n = 665)	*P*	Univariate regression		Multivariate regression		
				OR	*P*	Coefficient	*P*	OR (95% CI)
Male (n, %)	118(79.7)	513(77.1)	0.495	0.858(0.553–1.332)	0.495			
Age (year)	30.4 ± 9.1	32.3 ± 9.7	0.028	1.023(1.002–1.044)	0.029			
ALT (U/L)	63.0(40.0,107.0)	89.0(47.0,181.0)	0.001	1.005(1.003–1.008)	0.001			
AST (U/L)	46.0(34.0,65.0)	61.0(43.0,103.0)	0.001	1.015(1.009–1.021)	0.001	0.013	0.001	1.013(1.006–1.019)
GGT (U/L)	46.0(27.0,86.0)	81.0(48.0,141.0)	0.001	1.012(1.008–1.016)	0.001			
GGT (loge U/L)	3.5 ± 0.8	4.1 ± 0.8	0.001	2.124(1.667–2.707)	0.001			
Albumin (g/L)	46.4 ± 4.3	44.0 ± 5.1	0.001	0.911(0.877–0.946)	0.001	−0.063	0.002	0.939(0.901–0.978)
TBIL (μmol/L)	17.1 ± 8.5	19.0 ± 11.1	0.053	1.021(1.000–1.042)	0.054			
Creatinine (μmol/L)	83.0 ± 17.3	82.8 ± 28.0	0.929	1.000(0.993–1.006)	0.929			
FPG (mmol/L)	4.5 ± 0.8	4.5 ± 1.2	0.770	1.024(0.873–1.201)	0.770			
TC (mmol/L)	4.8 ± 1.1	4.4 ± 1.0	0.001	0.692(0.585–0.818)	0.001	−0.219	0.018	0.803(0.670–0.962)
TG (mmol/L)	1.2 ± 0.6	1.2 ± 0.6	0.458	0.892(0.659–1.207)	0.458			
HDL (mmol/L)	1.4 ± 0.4	1.3 ± 0.4	0.002	0.523(0.346–0.792)	0.002			
LDL (mmol/L)	2.6 ± 0.9	2.4 ± 0.8	0.003	0.704(0.572–0.866)	0.001			
WBC (10^9/L)	6.2 ± 1.5	5.8 ± 1.6	0.021	0.886(0.799–0.983)	0.023			
hemoglobin (g/L)	145.8 ± 18.5	142.6 ± 16.2	0.032	0.987(0.976–0.999)	0.032			
Platelet (10^9/L)	202.2 ± 49.3	182.6 ± 55.9	0.001	0.994(0.991–0.997)	0.001			
Platelet (loge 10^9/L)	5.3 ± 0.3	5.2 ± 0.3	0.001	0.262(0.139–0.494)	0.001	−1.075	0.003	0.341(0.169–0.691)
AFP (ng/mL)	3.9(2.5,7.0)	7.2(4.0,14.5)	0.001	1.009(1.001–1.016)	0.020			
AFP (loge ng/mL)	1.4 ± 0.9	2.1 ± 1.3	0.001	2.023(1.632–2.507)	0.001	0.466	0.001	1.593(1.264–2.007)
HBV DNA (lg IU/mL)	5.2 ± 2.3	4.8 ± 2.9	0.100	1.019(0.947–1.096)	0.612			
Constant						8.829	0.001	

Continuous variables were expressed as mean ± standard deviation or median (25th–75th percentiles) as appropriate.
SF: significant fibrosis (S2-4); ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transferase; TBIL: total bilirubin; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; WBC: white blood cell; AFP: α-fetoprotein.

Table 4
Diagnostic evaluation of the routine indexes for compensated cirrhosis and significant fibrosis detection in patients with hepatitis B.

Fibrosis	Index	Set	AUROC	Cutoff	Sen (%)	NPV (%)	NLR	Spe (%)	PPV (%)	PLR
Cirrhosis	HeBCI	Model	0.841	0.40	45.1	85.6	0.58	94.3	69.5	7.9
		Validating	0.779		41.8	88.9	0.63	92.0	50.6	5.22
		Model		0.09	92.3	95.5	0.16	46.9	33.4	1.74
		Validating			89.3	95.3	0.25	43.1	23.7	1.57
	FIB-4	Model	0.708	4.0	15.4	80.0	0.87	97.5	63.6	6.16
		Validating	0.690		14.6	84.8	0.91	94.3	33.3	2.56
		Model		0.52	96.7	92.2	0.29	11.3	23.9	1.09
		Validating			98.1	95.9	0.21	9.0	17.5	1.08
	APRI	Model	0.623	2.0	20.3	79.5	0.89	89.4	35.6	1.92
		Validating	0.595		22.3	84.9	0.90	86.4	24.5	1.64
		Model		1.0	52.8	82.2	0.75	62.9	29.1	1.42
		Validating			49.5	85.8	0.84	60.3	19.8	1.25
		Model		5.1	5.5	78.4	0.95	99.1	62.5	6.11
		Validating			5.83	83.9	0.98	96.6	25.0	1.71
		Model		0.3	97.8	88.6	0.45	4.9	22.9	1.03
		Validating			98.1	93.9	0.32	5.9	17.1	1.04
SF	HeBFI	Model	0.781	0.91	40.3	25.8	0.64	93.2	96.4	5.93
		Validating	0.776		39.7	17.3	0.66	90.8	91.2	4.32
		Model		0.60	93.5	49.4	0.23	28.4	85.4	1.31
		Validating			90.7	35.4	0.25	36.8	69.9	1.44
	APRI	Model	0.693	1.5	24.2	21.7	0.80	94.6	95.3	4.48
		Validating	0.729		26.6	15.0	0.79	93.4	96.7	4.03
		Model		0.5	83.2	34.1	0.43	39.2	86.0	1.37
		Validating			78.7	23.5	0.45	47.4	91.5	1.50
		Model		1.8	18.5	20.9	0.84	96.6	96.1	5.44
		Validating			20.0	14.3	0.83	96.1	97.3	5.13
		Model		0.3	96.7	37.1	0.38	8.8	82.6	1.06
		Validating			95.6	27.3	0.37	11.8	88.7	1.08
	FIB-4	Model	0.641	2.53	17.6	20.7	0.85	96.6	95.9	5.18
		Validating	0.641		19.7	13.9	0.86	93.4	95.6	2.98
		Model		0.5	95.3	32.8	0.33	14.2	83.0	1.11
		Validating			84.5	89.5	0.59	26.3	18.4	1.15

AUROC: the area under receiver operating characteristics curve; Sen: sensitivity; NPV: negative predictive value; NLR: negative likelihood ratio; Spe: specificity; PPV: positive predictive value; PLR: positive likelihood ratio; HeBCI: hepatitis B cirrhosis index; APRI: aspartate aminotransferase to platelet ratio index; FIB-4: fibrosis index based on the four indexes; SF: significant fibrosis; HeBFI: hepatitis B significant fibrosis index; SF: significant fibrosis.

were determined to have cirrhosis, and 545 (37.9%) patients with HeBCI < 0.09 were excluded from cirrhosis diagnosis. A total of 688 (47.8%) patients involved in the grey zone could, stepwise, undergo other non-invasive tests such as FIB-4, red cell distribution width-platelet ratio [15], and even liver biopsy to detect cirrhosis.

For detection of SF, the higher cut off 0.91 of HeBFI showed a PLR of 5.93, specificity 93.2% and PPV 96.4% in the model set and PLR 4.32, specificity 90.8% and PPV 91.2% in the validating set. The lower cut off 0.60 of HeBFI was characterised by an NLR of 0.23, sensitivity 93.5% and NPV 49.4% in the model set and NLR 0.25, sensitivity

90.7% and NPV 35.4% in the validating set. Therefore, 673 (46.8%) patients could be determined to be in a state of SF; among them, 514 (35.7%) patients with HeBFI > 0.91 were considered to have SF, with an accurate rate of 96.3%, and 159 (11.1%) patients with HeBFI < 0.60 were considered not to have SF. The remaining 765 patients involved in the grey zone could further, stepwise, undergo other routine indexes such as APRI and, if necessary, undergo liver biopsy to determine the state of SF.

A cut-off FIB-4 of 4.0, with highest PLR 6.16 for including in cirrhosis diagnosis in the model set was characterised by a decreased PLR lower to 2.56 in the validating set, implying that FIB-4 is of weak efficiency in cirrhosis diagnosis. However, a low cut-off of 0.52 provided a NLR 0.29 in the model set and 0.22 in the validating set with respective NPV 92.2% and 95.9%, suggesting that FIB-4 may be applied in excluding cirrhosis diagnosis. For SF detection, the cutoff 2.53 with highest PLR 5.18 in the model set was characterised by PLR 2.98 in the validating set; the NLR for excluding SF was 0.33 in the model set and 0.59 in the validating set, which implied that FIB-4 may not be valuable in SF detection.

A cut-off 2.0 for APRI had been recommended for detecting cirrhosis, which was characterised by PLR 1.92 in the model set and 1.64 in the validating set; another recommended cut-off of 1.0 excluded cirrhosis with NLR 0.75 in the model set and 0.84 in the validating set. These likelihood ratios would offer small and rarely important degrees of probability to diagnose cirrhosis [14]. Although an adjusted cutoff 5.1 provided a PLR 6.11 with moderate evidence in the model set, the PLR in the validating set decreased to 1.71; another adjusted lower cut-off of 0.3 excluded cirrhosis with NLR 0.45 in the model set and 0.32 in the validating set. As for SF, the recommended cut-off of 1.5 was characterised by PLR 4.48, 4.04 in the model set and the validating set, respectively, also providing small probability for diagnosis. While adjusting the cut-off up to 1.8, the corresponding PLR 5.74, 5.08 in both sets, would generate moderate diagnostic evidence. The recommended cut-off of 0.5 and adjusted cut-off of 0.3 for the APRI for excluding SF were characterised by NLR 0.43, 0.45 and 0.38, 0.37 in both sets, providing a small probability for excluding SF. Therefore, with adjusted cut-off of 1.8, APRI may only be applied in diagnosing SF with moderate statistical evidence.

3.5. *Risk stratification of cirrhosis and significant fibrosis, using HeBCI and HeBFI*

The classification tree method was used to explore the clinical utility of HeBCI and HeBFI for cirrhosis and SF (Fig. 2). According to HeBCI, the patients could be divided into five risk groups. The probability of cirrhosis was 1.80%, 7.05%, 15.04%, 42.24% and 75.90%, respectively, in these five groups in the model set (Fig. 2A). In the validation set, the probability was 2.52%, 8.33%, 13.4%, 21.26% and 60.66%, respectively (Fig. 2B).

According to HeBFI, the patients could also be divided into five risk groups. The probability of SF was 48.53%, 65.36%, 85.48%, 93.85% and 100.0%, respectively, in these five groups in the model set (Fig. 2C). In the validation set, the probability was 62.67%, 79.28%, 90.16%, 96.24% and 100.0%, respectively (Fig. 2D).

4. Discussion

In the present study, novel routine indexes for cirrhosis and SF detection were derived from routine variables in patients with CHB. Although HeBCI detected cirrhosis AUROCs 0.841 in the model set and 0.779 in the validation set, HeBFI detected SF with AUROCs with 0.781 in the model set and 0.776 in the validation set. The performances of HeBCI and HeBFI were significantly superior to that of FIB-4 and APRI in both model set and validation set. With HeBCI ≥ 0.4 and HeBCI < 0.09, 750 (52.2%) patients could be classified with cirrhosis or not with an 86% accuracy rate. With HeBFI ≥ 0.91 and HeBFI < 0.4, 673 (46.8%) patients could be classified as SF or non-SF with a 76.6% accuracy rate. As indexes consisting of routinely available variables of platelet, AST, GGT, AFP, albumin, TC and age, HeBCI and HeBFI could easily be applied as economical and convenient indexes to stratify the risk of significant fibrosis and cirrhosis, especially suitable to serve as outpatient screening tools to reduce the need of liver biopsy significantly in resource-limited settings. Although FIB-4 only showed a slight application in excluding cirrhosis diagnosis, APRI was only suggested to determine a minority of patients with SF. As derived from patients with HCV infection, FIB-4 and APRI may not be suitable for detecting significant fibrosis and cirrhosis in patients with CHB.

HeBCI and HeBFI consisted of routine variables of platelet, AST, GGT, AFP, albumin, TC and age. All these parameters had been demonstrated to be correlated with advanced fibrosis in previous studies [10,11,16–22]. Thrombocytopenia in liver disease might be partially due to the accumulation and destruction of platelets in the portal hypertensive-enlarged spleen, resulting from progressive liver fibrosis, and partially due to the impaired production of thrombopoietin [23]. However, ALT was exclusively cytoplasmic, and both mitochondrial and cytoplasmic forms of AST were found in all cells, suggesting that the AST elevation indicated hepatic injury and might result in fibrosis progression. Bile duct lesions caused by HBV and hepatitis C virus (HCV) infection could partially explain the elevated GGT; compared to patients with normal GGT, patients with elevated GGT often had significantly higher fibrosis scores [24]. AFP had been found to be correlated with hepatic impairment and chronic fibrosis; thus, AFP was helpful to differentiate fibrosis stages [25,26]. Albumin was exclusively synthesised in the liver; thus, the albumin level decreased along with the decline of hepatic synthetic function in patients with worsening liver fibrosis [27]. TC had been reported to be an independent influence factor of fibrosis by multivariate logistic regression analysis [16]. Age had been reported to be related to fibrosis due to the progression of fibrosis in CHB patients, and was therefore time-dependent [17,28]. In the current study, these parameters were confirmed as independent influence factors of fibrosis in multivariate logistic regression analysis.

FIB-4 and APRI have been recommended for evaluating and staging liver fibrosis in resource-limited settings by the World Health Organization in 2015 [12]. APRI has also been recommended as the preferred non-invasive test to assess the presence of cirrhosis, especially in resource-limited settings [29]. Several previous studies applying FIB-4 and APRI in detecting cirrhosis and significant fibrosis in CHB patients showed conflicting results: FIB-4 detected SF with AUROC 0.75–0.86 and cirrhosis with AUROC 0.77–0.93; these numbers were 0.63–0.86 for APRI [30–33]. In an international study of 2411 cases, APRI detected significant fibrosis and cirrhosis with AUROC 0.7, 0.76 in CHC, but only 0.64, 0.61 in CHB, respectively [32]. The AUROCs of APRI and FIB-4 in the current large cohort study were also lower than that in previous studies in patients with HCV infection [10,11]. However, the adj-AUROCs of APRI and FIB-4 were similar to that in previous studies of patients with CHB. FIB-4 was constructed in patients with human immunodeficiency virus/HCV co-infection, whereas APRI was derived from patients with HCV. HBV, HCV and human immunodeficiency virus had different influences on progression of fibrosis and clinical biomarkers. Therefore, the influence of aetiologies must be taken into account in assessing the diagnostic accuracy of non-invasive indexes. Actually, a previous study in 2005, by Wai et al. [34], indicated that models with non-invasive markers in predicting histology from chronic hepatitis C patients were unsuitable for CHB patients. The AUROCs of APRI and FIB-4 detecting cirrhosis in the current study were slightly lower than recent multicentre studies (0.65 and 0.74,

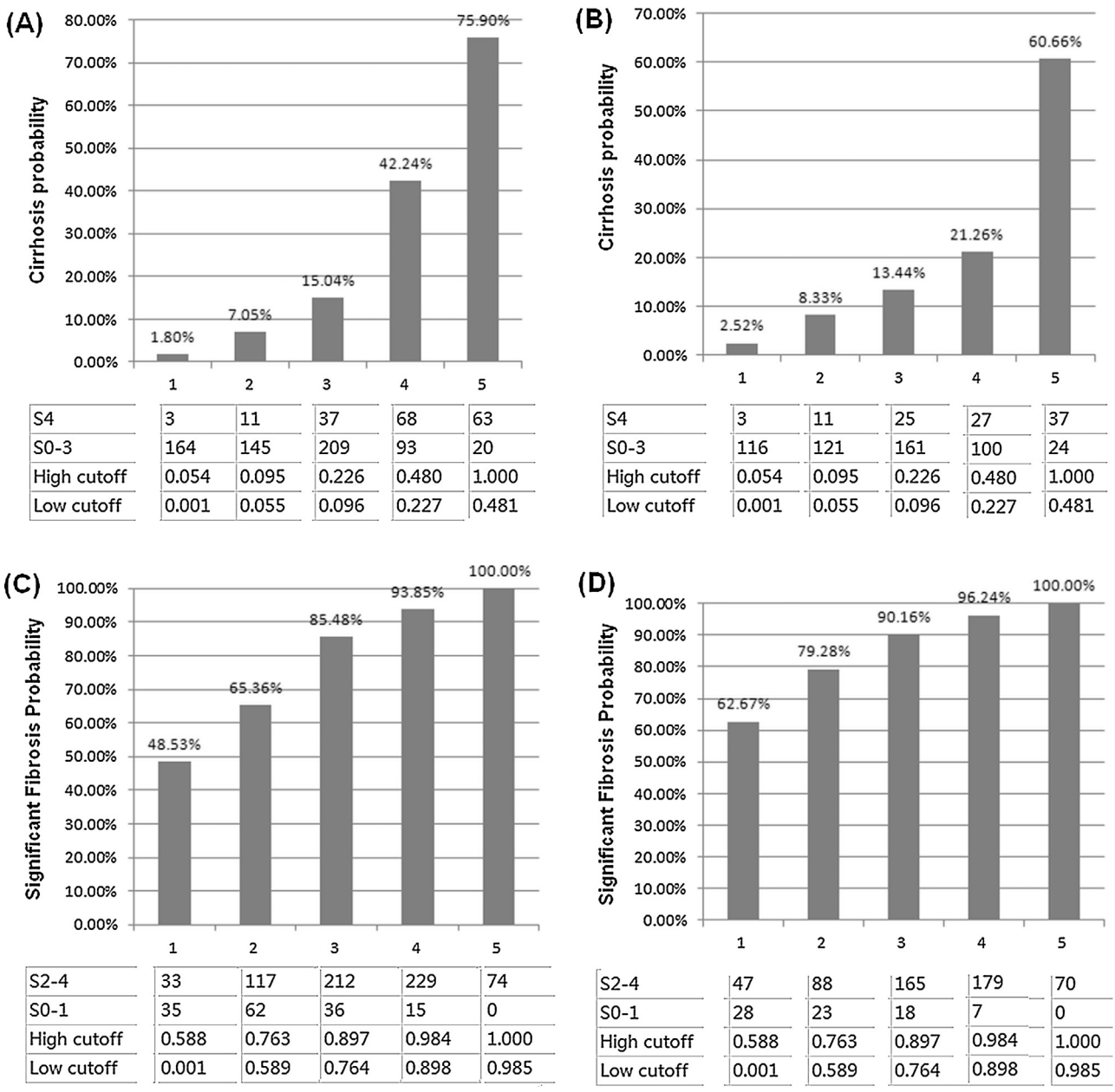

Fig. 2. Risk stratification of liver fibrosis by routinely available indexes, according to optimal cut-off values determined by classification tree method. HeBCI for cirrhosis detection in model set (A) and validation set (B); HeBFI for significant fibrosis detection in model set (C) and validation set (D). **HeBCI**: hepatitis B cirrhosis index; **HeBFI:** hepatitis B significant fibrosis index.

respectively) [35], which may be due to lower prevalence of cirrhosis in the present study. The analysis of the large cohort of CHB patients in the present study indicated that although FIB-4 may be applied in excluding cirrhosis diagnosis, APRI may be valuable in detecting SF but inefficient in diagnosing cirrhosis.

The present study was characterised by several strengths. First, the present large-cohort study included 1438 antivirus treatment-naïve CHB patients and provided a convincing conclusion for assessment of diagnostic accuracy. Second, the present study included only patients with a liver biopsy sample length $\geq$15 mm, which would significantly improve the accuracy of the gold standard of liver histological evaluation. Third, the AUROCs in the current study were adjusted using the DANA method to balance the influence of different prevalences of fibrosis stages, providing standard results for further comparisons in different study populations. Last, all parameters were readily obtained in routine examinations and did not incur extra cost.

Of course, several limits should also be noted in the present study. First, the present study was a single-centre study, which might reduce the representativeness of the study population and thus result in potential sample selection bias, but the cohort was large enough to offset this flaw. Second, as a retrospective study, alcohol consumption was not applied in the current study. However, because patients diagnosed with alcoholic liver disease had been excluded from the final analysis, the influence from the patients' characteristics may be ignored. Third, the fibrosis stages were diagnosed according to the Scheuer system and not the more routinely used Ishak and METAVIR systems. This pitfall was due to the retrospective characteristics of the present study. In the past 10 years, liver histological evaluation was classified by the Scheuer system in our hospital. However, considering the similar-

ity between the descriptions of significant fibrosis (enlargement of portal tract with rare septa formation in METAVIR and peri-portal or portal–portal septa but intact architecture in Scheuer), the diversity may be ignored.

Conflict interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.07.001.

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