

Accuracy of FibroScan in analysis of liver fibrosis in patients with concomitant chronic Hepatitis B and nonalcoholic fatty liver disease

Qiang Li, MD*, Chenlu Huang, MD, Wei Xu, MD, Qiankun Hu, MD, Liang Chen, MD*

Abstract

Liver steatosis could affect the accuracy of FibroScan in patients with chronic hepatitis B (CHB) and nonalcoholic fatty liver disease (NAFLD). This study aimed to assess the accuracy and cut-off values of FibroScan for diagnosing liver fibrosis and cirrhosis in patients with concomitant CHB and NAFLD.

A total of 116 patients with concomitant CHB and NAFLD who underwent FibroScan test and liver biopsy were retrospectively enrolled. Liver fibrosis was staged according to the METAVIR scoring system. Calculations of the areas under receiver-operating characteristic curves (AUROC) were performed and compared for the staging of liver fibrosis.

The AUROCs for FibroScan, gamma-glutamyl transpeptidase to platelet ratio (GPR), aspartate aminotransferase to platelet ratio index (APRI), fibrosis index based on 4 factors (FIB-4), and NAFLD Fibrosis Score (NFS) were 0.87, 0.73, 0.69, 0.57, and 0.57 for the diagnosis of significant liver fibrosis (METAVIR \geq F2); 0.89, 0.77, 0.75, 0.68, and 0.60 for severe liver fibrosis (METAVIR \geq F3); and 0.94, 0.86, 0.80, 0.74, and 0.63 for cirrhosis (F4), respectively. The cutoff values of FibroScan for staging liver fibrosis with sensitivity at least 90% were: 8.0 kPa for significant liver fibrosis, and 10.5 kPa for cirrhosis. The cutoff values of FibroScan for staging liver fibrosis with specificity at least 90% were: 10.8 kPa for significant liver fibrosis, and 17.8 kPa for cirrhosis.

FibroScan provides high value for the diagnosis of liver fibrosis and cirrhosis in patients with concomitant CHB and NAFLD.

Abbreviations: ALT = alanine aminotransferase, APRI = aspartate aminotransferase to platelet ratio index, AST = aspartate aminotransferase, AUROC = area under the receiver operating characteristic curve, BMI = body mass index, CHB = chronic hepatitis B, FIB-4 = fibrosis index based on 4 factors, GGT = gamma-glutamyl transpeptidase, GPR = gamma-glutamyl transpeptidase to platelet ratio, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HDV = hepatitis D virus, HIV = human immunodeficiency virus, LSM = liver stiffness measurement, NAFLD = nonalcoholic fatty liver disease, NFS = NAFLD fibrosis score, NPV = negative predictive value, PPV = positive predictive value, ROC curve = receiver-operating characteristic curve.

Keywords: chronic hepatitis B, FibroScan, liver fibrosis, liver stiffness measurement, nonalcoholic fatty liver disease

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QL, CH, and WX contributed equally in this study.

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1. Introduction

Chronic hepatitis B virus (HBV) infection is one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC) in China.^[1] Nonalcoholic fatty liver disease (NAFLD) is also a common disease that affects 20% to 40% of the general population.^[2] In recent years, the number of patients with concomitant CHB and NAFLD is increasing gradually. A study performed by Bondini et al^[3] reported that the prevalence of NAFLD was 20% in patients with CHB. The mortality of patients with concomitant CHB and NAFLD is related to the development of liver fibrosis and cirrhosis, which can progress to HCC, liver function de-compensation, and liver failure.^[4] Therefore, it is very necessary to distinguish liver fibrosis and cirrhosis for optimization of therapy, evaluation of prognosis, and prevention of disease progression in patients with concomitant CHB and NAFLD.

Liver biopsy is considered the criterion standard for assessment of liver fibrosis and cirrhosis. However, it is not routinely performed due to its invasiveness, cost, and potential complications.^[5] In recent years, new noninvasive techniques have been developed to assess the degree of liver fibrosis. Of these techniques, FibroScan has been the most widely used. Numerous studies have confirmed the efficiency of FibroScan in the diagnosis of liver fibrosis and cirrhosis in patients with CHB.^[6–8] However, the concomitant existence of NAFLD raises issues and challenges for the clinical applications of FibroScan in patients with CHB. Because major components of NAFLD such

as obesity,^[9] liver steatosis,^[10] and liver inflammation may affect the liver stiffness measurement (LSM) values evaluated by FibroScan. The increased fat in patients with NAFLD might lead to a poorer transmission of the ultrasound when FibroScan measurements were performed, leading to unreliable FibroScan results.^[11]

FibroScan is a reliable tool for the diagnosis of liver fibrosis and cirrhosis in patients with CHB, patients with hepatitis C, and patients with NAFLD.^[12] However, the use of FibroScan has not mentioned for patients with concomitant CHB and NAFLD in current guidelines.^[1,2,13] For patients with concomitant CHB and NAFLD, it is necessary to question whether FibroScan would be more or less effective for the diagnosis of liver fibrosis. Therefore, this study aimed to evaluate the accuracy of FibroScan for the staging of liver fibrosis in patients with concomitant CHB and NAFLD.

2. Patients and methods

2.1. Patients

We retrospectively enrolled 184 consecutive patients with concomitant CHB and NAFLD from Shanghai Public Health Clinical Center, a tertiary hospital in Shanghai, China, between January 2013 and January 2019. CHB was diagnosed as the persistent positivity of serum HBsAg and/or HBV DNA for >6 months.^[14] NAFLD was diagnosed as at least 5% biopsy-proven hepatic steatosis without significant alcohol consumption. No significant alcohol consumption was defined as alcohol consumption less than 20 g/day and history of drinking <5 years. The inclusion criteria were: serum HBsAg and/or HBV DNA positivity for >6 months; biopsy-proven NAFLD; underwent routine laboratory testing, FibroScan, and liver biopsy. The exclusion criteria were: alcohol consumption >20 g/day for >5 years (n=20), previous or current antiviral therapy (n=5), hepatitis C virus (HCV), hepatitis D virus (HDV), or human immunodeficiency virus (HIV) co-infection (n=9), combined with autoimmune liver disease (n=1), inappropriate biopsy samples (n=5), failure of FibroScan measurement (n=10), and unreliable LSM values (n=18). Finally, 116 patients with concomitant CHB and NAFLD were included in this study.

This study was approved by the ethics board of Shanghai Public Health Clinical Center. The informed consent for FibroScan (non-medicare test in China) and liver biopsy (invasive test) had been obtained as part of routine clinical practices. The medical data could be used for further studies and were also obtained as part of the written informed consent.

2.2. Liver histological examination

Because of its invasiveness and potential complications, liver biopsy was suggested when the noninvasive tests could not provide enough information for the causes and/or severity of liver injury and fibrosis. In this retrospective study, liver biopsy was recommended by physicians based on the comprehensive evaluation of age, alanine aminotransferase (ALT) levels, HBV DNA levels, the course of liver disease, family history of cirrhosis and HCC, and the LSM values of FibroScan tests with full respect for the wishes of the patients.

Liver biopsy was performed within 1 week after enrollment. Liver biopsies were fixed in formalin, embedded in paraffin, and a minimum of 15 mm of liver tissue with at least 6 portal tracts was considered suitable for histopathological analysis.^[15] The double

examination for liver biopsy specimens routinely was done in clinical practice in our hospital. Therefore, all liver biopsy specimens were routinely analyzed by 2 pathologists, and were further reviewed by a third senior pathologist if discrepant readings occurred. Liver fibrosis was staged according to the METAVIR scoring system^[16]: F0, absence of fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; F4, cirrhosis. Liver steatosis was staged according to the NASH Clinical Research Network scoring system^[17]: S0, <5%; S1, 5% to 33%; S2, 34% to 66%; and S3, >67%. Significant liver fibrosis, severe liver fibrosis, and cirrhosis were defined as METAVIR fibrosis score \geq F2, \geq F3, and F4, respectively.

2.3. Liver stiffness measurement

In clinical practice, the FibroScan tests were firstly recommended to evaluate liver fibrosis and cirrhosis in patients with CHB and NAFLD because of its noninvasive nature and relatively high diagnostic performance. Transient elastography examinations were performed by trained operators according to the manufacturers' recommendations using the standard probe (M probe) 1 to 3 days before liver biopsy.^[18] The LSM values were considered reliable when 10 valid examinations were obtained with the maximum number of attempts set at 20. The FibroScan evaluation was considered as unreliable when IQR/LSM was >0.30 in patients with LSM \geq 7.1 kPa.^[19]

2.4. Noninvasive serum fibrosis models calculation

Fasting blood samples were obtained, and routine laboratory tests were performed 1 to 3 days before liver biopsy. The gamma-glutamyl transpeptidase (GGT) to platelet ratio (GPR), aspartate aminotransferase (AST) to platelet ratio index (APRI), and fibrosis index based on 4 factors (FIB-4) have been widely adopted for evaluation of liver fibrosis in patients with CHB.^[15] The NAFLD Fibrosis Score (NFS) has been proposed to evaluate liver fibrosis in patients with NAFLD.^[15] Therefore, GPR, APRI, FIB-4, and NFS were selected as serum fibrosis models to compare with FibroScan.

- (1) $GPR = (GGT [IU/L]/ULN \text{ of } GGT)/\text{platelet count } (10^9 \text{ cells/L}) \times 100.$ ^[20]
- (2) $APRI = (AST [IU/L]/ULN \text{ of } AST)/\text{platelet count } (10^9 \text{ cells/L}) \times 100.$ ^[21]
- (3) $FIB-4 = (\text{age [years]} \times AST [IU/L]) / (\text{platelet count } [10^9 \text{ cells/L}] \times (ALT [IU/L])^{1/2}).$ ^[22]
- (4) $NFS = (-1.675 + 0.037 \times \text{age [years]} + 0.094 \times \text{BMI [kg/m}^2\text{]} + 1.13 \times \text{impaired fasting glucose (IFG)/diabetes (yes=1, no=0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet count } (10^9/\text{L}) - 0.66 \times \text{albumin [g/dL]}).$ ^[15] IFG was diagnosed when a participant did not have diabetes, but had a fasting blood glucose of 5.6 to <7.0 mmol/L.

2.5. Statistical analysis

The normality test was performed for continuous variables using the Kolmogorov-Smirnov test. Normal distribution variables, non-normal distribution continuous variables, and categorical variables were shown as means \pm standard deviations, medians and interquartile ranges (IQRs), and counts (percentage), respectively. The diagnostic performances were assessed by

correlating noninvasive tests results and liver biopsy results using the Spearman correlation coefficient, and building the receiver-operating characteristics (ROC) curves. Areas under the ROC curves (AUROCs) of noninvasive tests were calculated for the diagnosis of significant liver fibrosis, severe liver fibrosis, and cirrhosis. The AUROC values were compared using the Delong test.^[2,3] Two sets of cutoff values were calculated respectively: sensitivity $\geq 90\%$, specificity $\geq 90\%$. All significance tests were 2-tailed, and $P \geq .05$ was considered no significant difference between 2 groups/methods. Statistical analysis was performed using SPSS 15.0 (SPSS Inc, Chicago, IL) and MedCalc 16.1 (MedCalc Software bvba, Ostend, Belgium).

3. Results

3.1. Patient characteristics

Patient characteristics are shown in Table 1. In this study, 66.4% were male, 79.3% were HBeAg-positive, and median age of enrolled patients was 36 years. The median HBV DNA, ALT, AST, GGT, body mass index (BMI), and LSM values were 7.5 log₁₀ copies/mL (IQR 6.9–7.7), 51 IU/L (IQR 34–78), 29 IU/L (IQR 23–40), 25 IU/L (IQR 15–72), 25.5 kg/m² (IQR 22.4–28.4), and 8.7 kPa (IQR 5.4–12.8), respectively.

Table 1
Baseline characteristics of the study population.

	Enrolled patients (n = 116)
Male, n (%)	77 (66.4%)
Age, y, n (%)	36 ± 10
>40	32 (27.6%)
30–40	48 (41.4%)
<30	36 (31.0%)
HBeAg positive, n (%)	92 (79.3%)
Diabetes, n (%)	15 (12.9%)
IFG, n (%)	42 (36.2%)
HBV DNA (log ₁₀ copies/mL)	7.5 (6.9 to 7.7)
>6 log ₁₀ copies/mL, n (%)	100 (86.2%)
3–6 log ₁₀ copies/mL, n (%)	16 (13.8%)
Alanine aminotransferase, IU/L	51 (34 to 78)
<1 ULN, n (%)	37 (31.9%)
1–2 ULN, n (%)	52 (44.8%)
>2 ULN, n (%)	27 (23.3%)
Aspartate aminotransferase, IU/L	29 (23 to 40)
Gamma-glutamyl transpeptidase, IU/L	25 (15 to 72)
Albumin, g/L	45 ± 4
Total bilirubin, μmol/L	13 (10 to 18)
Platelet count (10 ⁹ cells/L)	196 ± 61
Body mass index, kg/m ²	25.5 (22.4 to 28.4)
Liver stiffness measurement, kPa	8.7 (5.4 to 12.8)
GPR	0.30 (0.16 to 0.76)
APRI	0.39 (0.30 to 0.56)
FIB-4	0.75 (0.56 to 1.14)
NFS	−2.76 (−3.50 to −1.94)
Immune tolerance phase, n (%)	31 (26.7%)
Immune clearance phase, n (%)	61 (52.6%)
Inactive carrier phase, n (%)	3 (2.6%)
Reactivation of HBeAg-negative phase, n (%)	21 (18.1%)

APRI = aspartate aminotransferase to platelet ratio index, FIB-4 = fibrosis index based on 4 factors, GPR = gamma-glutamyl transpeptidase to platelet ratio index, IFG = impaired fasting glucose, GPR = gamma-glutamyl transpeptidase to platelet ratio index, ULN = upper limit of normal (the ULN of alanine aminotransferase is 40 IU/L). Normal distribution variables, non-normal distribution continuous variables, and categorical variables, were showed as means ± standard deviations, medians (IQR), and counts (percentage), respectively.

The percentages of patients >40 years, 30 to 40 years, and <30 years were 27.6%, 41.4%, and 31.0%, respectively. The percentages of patients with HBV DNA >6 log₁₀ copies/mL, and 3 to 6 log₁₀ copies/mL, were 86.2% and 13.8%, respectively. Among the 116 enrolled patients, 37 (31.9%) had normal ALT levels, 52 (44.8%) had mildly elevated ALT levels (1–2 upper limit of normal [ULN]), and 27 (23.3%) had significantly elevated ALT levels (>2 ULN). The ULN of ALT is 40 IU/L in this study.

3.2. Liver histologic results

The liver histological results of enrolled patients are shown in Table 2. The liver inflammation stages were as follows: A0 = 7 (6.0%); A1 = 13 (11.2%); A2 = 51 (44.0%); and A3 = 45 (38.8%). The liver fibrosis stages were as follows: F0 = 19 (16.4%); F1 = 49 (42.2%); F2 = 23 (19.8%); F3 = 13 (11.2%); and F4 = 12 (10.3%). The hepatic steatosis stages were as follows: S1 = 76 (65.5%); S2 = 33 (28.4%); and S3 = 7 (6.0%). Of 116 patients with concomitant CHB and NAFLD, 48 (41.4%), 25 (21.6%), and 12 (10.3%) were classified as having significant liver fibrosis, severe liver fibrosis, and cirrhosis, respectively.

3.3. Correlations between noninvasive fibrosis tests and histological fibrosis stages

Correlations between noninvasive fibrosis tests and histological fibrosis stages are shown in Table 3. FibroScan ($r = 0.67$, $P < .001$), GPR ($r = 0.44$, $P < .001$), and APRI ($r = 0.34$, $P < .001$) demonstrated a correlation with liver histological fibrosis stages. The correlation between FibroScan tests and liver histological fibrosis stages was significantly superior to that between serum fibrosis models and liver histological fibrosis stages.

3.4. Pairwise comparison for diagnostic performances of noninvasive fibrosis tests

ROC curves of noninvasive fibrosis tests for the diagnosis of significant liver fibrosis, severe liver fibrosis, and cirrhosis are shown in Figure 1. Pairwise comparisons of AUROC values were

Table 2
Liver histological results of the study population.

	Total (n = 116)
METAVIR liver inflammation stage	
A0	7 (6.0%)
A1	13 (11.2%)
A2	51 (44.0%)
A3	45 (38.8%)
METAVIR liver fibrosis stage	
F0	19 (16.4%)
F1	49 (42.2%)
F2	23 (19.8%)
F3	13 (11.2%)
F4	12 (10.3%)
Hepatic steatosis stage	
S1	76 (65.5%)
S2	33 (28.4%)
S3	7 (6.0%)
Significant liver fibrosis	48 (41.4%)
Sever liver fibrosis	25 (21.6%)
Cirrhosis	12 (10.3%)

Table 3**Correlations between noninvasive fibrosis tests and liver histological fibrosis stages.**

Variables	Spearman <i>r</i>	<i>P</i>
FibroScan	0.67	<.001
GPR	0.44	<.001
APRI	0.34	<.001
FIB-4	0.09	.338
NFS	0.08	.398

APRI = aspartate aminotransferase to platelet ratio index, FIB-4 = fibrosis index based on 4 factors, GPR = gamma-glutamyl transpeptidase to platelet ratio index, NFS = NAFLD Fibrosis Score, Spearman *r* = correlation coefficient.

presented in Table 4. For the diagnosis of significant liver fibrosis, FibroScan had a significantly better diagnostic performance than GPR, APRI, FIB-4, and NFS. Similarly, for severe liver fibrosis, FibroScan had a significantly better diagnostic performance than GPR, APRI, FIB-4, and NFS (AUROC of 0.89, 0.77, 0.75, 0.68, and 0.60 for FibroScan, GPR, APRI, FIB-4, and NFS, respectively; all *P* < .05). For cirrhosis, FibroScan also had a significantly better diagnostic performance than GPR, APRI, FIB-4, and NFS.

3.5. Diagnostic thresholds of FibroScan

The diagnostic thresholds of FibroScan are shown in Table 5. The cutoff values of FibroScan for ruling out diseases with at least 90% sensitivity were: 8.0 kPa for significant liver fibrosis (the sensitivity, specificity, PPV, and NPV was 92%, 79%, 69%, and 92%, respectively), and 10.5 kPa for cirrhosis (the sensitivity, specificity, PPV, and NPV was 92%, 84%, 39%, and 98%, respectively). The cutoff values for ruling in diseases with a specificity of at least 90% were: 10.8 kPa for significant liver fibrosis (the sensitivity, specificity, PPV, and NPV was 42%, 91%, 77%, and 69%, respectively), and 17.8 kPa for cirrhosis (the sensitivity, specificity, PPV, and NPV was 75%, 90%, 47%, and 96%, respectively).

4. Discussion

FibroScan is a noninvasive test to estimate liver fibrosis and cirrhosis in patients with CHB,^[24] however the diagnostic performance is affected by several factors including ALT

Table 4**The AUROCs of noninvasive fibrosis tests.**

	Significant fibrosis AUROC (95% CI)	Severe fibrosis AUROC (95% CI)	Cirrhosis AUROC (95% CI)
FibroScan	0.87 (0.79–0.92)	0.89 (0.82–0.94)	0.94 (0.88–0.98)
GPR	0.73 (0.64–0.81)	0.77 (0.68–0.84)	0.86 (0.79–0.92)
APRI	0.69 (0.60–0.77)	0.75 (0.66–0.82)	0.80 (0.72–0.87)
FIB-4	0.57 (0.48–0.66)	0.68 (0.58–0.76)	0.74 (0.65–0.82)
NFS	0.57 (0.48–0.66)	0.60 (0.50–0.69)	0.63 (0.54–0.72)
FibroScan vs GPR	<i>P</i> = .023	<i>P</i> = .036	<i>P</i> = .045
FibroScan vs APRI	<i>P</i> = .006	<i>P</i> = .028	<i>P</i> = .016
FibroScan vs FIB-4	<i>P</i> < .001	<i>P</i> < .001	<i>P</i> = .039
FibroScan vs NFS	<i>P</i> < .001	<i>P</i> < .001	<i>P</i> < .001

APRI = aspartate aminotransferase to platelet ratio index, AUROC = the area under receiver-operating characteristic curve, FIB-4 = fibrosis index based on 4 factors, GPR = gamma-glutamyl transpeptidase to platelet ratio index, NFS = NAFLD Fibrosis Score.

flares,^[25] BMI,^[9] and hepatic steatosis.^[10] A study of 170 patients with CHB demonstrated that hepatic steatosis was independently related to the severity of liver histological fibrosis.^[26] Petta et al^[27] found that patients with liver steatosis had higher LSM values measured by FibroScan, which led to overestimations of the severity of liver fibrosis. So far, for diagnosis of liver fibrosis in patients with CHB, whether FibroScan would be interfered by the co-occurrence of NAFLD is not clear. The diagnostic performances and corresponding cutoff values of FibroScan for liver fibrosis and cirrhosis is unclear in patients with concomitant CHB and NAFLD.

In this retrospective cohort of 116 patients with concomitant CHB and NAFLD, FibroScan performed well for the diagnosis of different stages of liver fibrosis. The AUROC of FibroScan was 0.87 for the diagnosis of significant liver fibrosis, 0.89 for severe liver fibrosis, and 0.94 for cirrhosis, suggesting that FibroScan enabled accurate evaluation of liver fibrosis in patients with coexisting CHB and NAFLD. The reliability of FibroScan for the detection of fibrosis in NAFLD and chronic viral hepatitis had been evaluated in other studies.^[28,29] Gaia et al^[28] confirmed that FibroScan can be considered a valid support to detect fibrosis in chronic liver disease related to HCV but it should be interpreted cautiously in CHB and NAFLD patients, where host or disease-related factors may modify its accuracy.

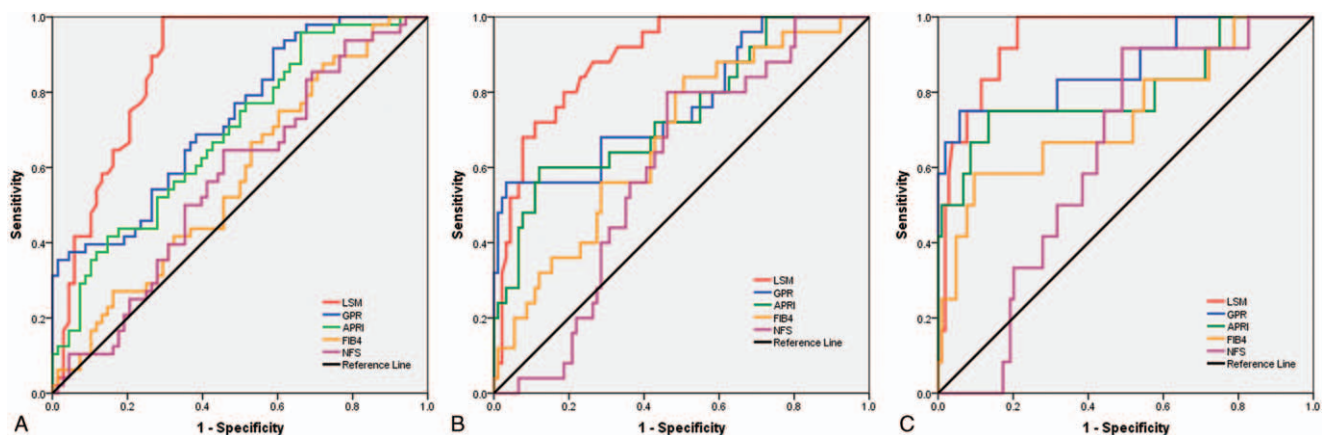


Figure 1. ROC curves of noninvasive fibrosis tests for the diagnosis of significant liver fibrosis (A), severe liver fibrosis (B), and cirrhosis (C). APRI = aspartate transaminase to platelet ratio, FIB-4 = fibrosis index based on 4 factors, GPR = gamma-glutamyl transpeptidase to platelet ratio, LSM = liver stiffness measurement, NFS = NAFLD Fibrosis Score, ROC = receiver-operating characteristic curve.

Table 5
Diagnostic thresholds of FibroScan.

	Cut-offs	Se (%)	Sp (%)	PPV (%)	NPV (%)	+LR	-LR
Significant liver fibrosis	8.0*	92	79	69	92	3.12	0.12
	10.8†	42	91	77	69	4.72	0.64
Cirrhosis	10.5*	92	84	39	98	5.61	0.10
	17.8†	75	90	47	96	7.80	0.28

+LR=positive likelihood ratio, -LR=negative likelihood ratio, NPV=negative predictive value, PPV=positive predictive value, Se=sensitivity, Sp=specificity.

*Cut-offs according to at least 90% sensitivity.

†Cut-offs according to at least 90% specificity.

The performance of FibroScan in the assessment of liver fibrosis in patients with hepatitis C has been examined in numerous studies.^[30,31] In comparison, relatively few studies are dedicated to FibroScan in subjects with HBV. Cardoso et al found that in HBV patients, FibroScan measurement accurately predicts the absence or presence of significant fibrosis, advanced fibrosis or cirrhosis, and shows similar performances as compared to HCV patients ($P=.975$, $P=.820$, $P=.740$, respectively).^[32] Marcellin et al^[24] also found that FibroScan appears to be reliable for detection of significant fibrosis or cirrhosis in patients with hepatitis B and cutoff values are only slightly different from those observed in patients with hepatitis C. According to the WHO guidelines on the treatment of patients with CHB, the cutoff values for FibroScan were 7 to 8.5 kPa for the diagnosis of significant liver fibrosis and 11 to 14 kPa for the diagnosis of cirrhosis, respectively.^[33] In this study, the cutoff values of FibroScan for ruling in disease were 10.8 kPa for significant liver fibrosis and 17.8 kPa for cirrhosis, respectively.

The strength of this study is that we not only demonstrated the good performance of FibroScan for the diagnosis of liver fibrosis in patients with coexisting CHB and NAFLD, but also determined the specific cutoff values of FibroScan to identify significant liver fibrosis and cirrhosis in patients with concomitant CHB and NAFLD. Indeed, the major challenge using FibroScan in patients with NAFLD is the lower measurement success rate in obese patients. Once the measurement is successful, the LSM values could evaluate accurately liver fibrosis and cirrhosis in patients with concomitant CHB and NAFLD.

According to guidelines,^[1,2] immune tolerant phase is characterized by the presence of serum HBeAg, very high levels of HBV DNA and ALT persistently within the normal range; immune clearance phase is characterized by the presence of serum HBeAg, high levels of HBV DNA, and elevated ALT; inactive carrier phase is characterized by the presence of serum antibodies to HBeAg (anti-HBe), undetectable or low (<2000 IU/mL) HBV DNA levels and normal ALT; reactivation of HBeAg-negative phase is characterized by the lack of serum HBeAg, moderate to high levels of serum HBV DNA and elevated ALT values. In this study, subjects with HBeAg positive, HBV DNA >10⁵ copies/mL, and ALT ≤40 IU/mL were classified as immune tolerant phase; subjects with HBeAg positive, HBV DNA >10⁵ copies/mL, and ALT >40 IU/mL were classified as immune clearance phase; subjects with anti-HBe positive, HBV DNA <10⁴ copies/mL, and ALT ≤40 IU/mL were classified as inactive carrier phase; subjects with HBeAg negative, HBV DNA >10³ copies/mL, and ALT >40 IU/mL were classified as reactivation of HBeAg-negative phase. Based on the grouping criterion, in this study, 31 (26.7%), 61 (52.6%), 3 (2.6%), and 21 (18.1%) were classified as having immune tolerance phase, immune clearance phase, inactive

carrier phase, and reactivation of HBeAg-negative phase, respectively. The enrollment of skewed patient groups could be explained as follows. According to the clinical guidelines on the treatment of CHB, patients with HBV DNA >20,000 IU/mL and ALT >2 ULN can start treatment even without a liver biopsy.^[1] Therefore, a considerable proportion of the patients with high ALT and HBV DNA levels who might be in immune clearance phase or reactivation of HBeAg-negative phase were not enrolled because they started antiviral therapy without liver biopsy tests. Some patients with HBeAg-negative, undetectable or low (<2000 IU/mL) HBV DNA levels and normal ALT levels who were in inactive carrier phase, usually had no indication for liver biopsy, and were not enrolled because they had no liver biopsy tests.^[1]

According to the WHO guidelines on the treatment of patients with CHB, the limitations with FibroScan include the following situation: it uses a single cut-off and therefore reported sensitivities and specificities of FibroScan may be overestimated across fibrosis stages.^[33] Therefore, in this study, FibroScan uses 2 cutoff points for the diagnosis of specific fibrosis stages, as the use of a single cut-off would result in suboptimal sensitivity and specificity.^[33] A high cut-off with high specificity is used to diagnose persons with a particular stage of fibrosis, and a low cut-off with high sensitivity to rule out the presence of a particular stage of fibrosis.^[33] Adolescent et al^[34] also found that a dual cut-off algorithm allowed for correctly classifying both significant fibrosis and cirrhosis in the majority of the patients with CHB, independent of ALT values, thus reducing the need for liver biopsy investigations. Although the WHO guidelines recommend APRI as the preferred noninvasive test to assess the presence of cirrhosis in resource-limited settings,^[33] in this study, the GPR showed better accuracy for the diagnosis of significant liver fibrosis, severe liver fibrosis, and cirrhosis. The results were in agreement with previous studies performed by Lemoine et al,^[20] Li et al,^[35] and Cai et al,^[36] in which GPR not only yielded good AUROCs for predicting significant fibrosis and cirrhosis but also showed better performance compared with APRI.

In this study, the cutoff values of FibroScan for the diagnosis of significant hepatic fibrosis were higher than other reports in CHB.^[24,32] The possible reasons were as follows. First, this study enrolled many patients in immune tolerance stage of CHB (26.7%), who were considered to be at low risk of liver fibrosis and cirrhosis. The enrollment of skewed patient groups might lead to the difference in prevalence of fibrosis and cirrhosis, and then lead to the different cutoff values of FibroScan in the studied populations, known as the spectrum bias.^[37,38] Second, the existence of NAFLD might increase the liver inflammation levels, and affected the cutoff values of FibroScan. One limitation of

FibroScan is that the LSM values increase with hepatic necroinflammatory levels regardless of the fibrosis stage. Verveer et al demonstrated that hepatic inflammation assessed by hepatic necroinflammatory index (Ishak) increased LSM values regardless of fibrosis stage ($P < .001$).^[39]

This study has several limitations. First, all enrolled patients in this study came from a tertiary hospital for the management of chronic liver disease, and it is uncertain whether the results would be influenced by a selection bias. Second, this study is a retrospective, single-center study, and the sample size is small. Large-scale, multicenter, prospective cohort studies are needed for further evaluation of the clinical use of FibroScan in patients with concomitant CHB and NAFLD.

In conclusion, this study confirmed that FibroScan is a valuable diagnostic tool for liver fibrosis and cirrhosis in patients with coexisting CHB and NAFLD. The cutoff values of FibroScan for ruling in disease were: 10.8 kPa for significant liver fibrosis, and 17.8 kPa for cirrhosis, respectively. It is important to note that most patients in this study were in immune tolerance phase and immune clearance phase of CHB; therefore, the cutoff values of FibroScan should be interpreted with caution and further validated in other clinical phases of CHB in a cohort with large sample size.

Author contributions

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