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Original Article

Optimal use of red cell volume distribution width-to-platelet ratio to exclude cirrhosis in patients with chronic hepatitis B☆

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ABSTRACT

Background and aims: Hepatitis B virus (HBV) infection is a major public health issue worldwide as it may cause serious liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). Ruling out cirrhosis is important when treating chronic hepatitis B (CHB). The aim of this study was to compare the performance of the aspartate aminotransferase-to-platelet ratio index (APRI), fibrosis score based on four factors (FIB-4), and red cell volume distribution width-to-platelet ratio (RPR) in diagnosing liver fibrosis stages and to identify new cut-off values to rule out cirrhosis.

Methods: Between 2005 and 2020, 2182 eligible individuals who underwent liver biopsy were randomly assigned to derivation and validation cohorts in a 6:4 ratio. A grid search was applied to identify optimal cut-off values with a sensitivity of >90% and a negative predictive value (NPV) of at least 95%.

Results: Overall, 1309 individuals (175 patients with cirrhosis) were included in the derivation dataset, and 873 (117 patients with cirrhosis) were included in the validation cohort. The area under the receiver operating characteristic curve of RPR for diagnosing cirrhosis was 0.821, which was comparable to that of APRI (0.818, $P = 0.7905$) and FIB-4 (0.803, $P = 0.2395$). When applying an RPR of 0.06, cirrhosis was correctly identified with a sensitivity of 93.1% and an NPV of 97.1%, while it misclassified 12 of 175 (6.9%) patients in the derivation cohort. In the validation cohort, RPR had a sensitivity and NPV of 97.4% and 99.0%, respectively, and only misclassified 3 of 117 (2.6%) patients. Subgroup analysis indicated that the new RPR cut-off value performed more consistently than that of APRI and FIB-4 in all subgroups.

Conclusion: A recently established cut-off value for RPR (≤0.06) was validated and was more effective than APRI and FIB-4 in excluding patients with cirrhosis due to a higher sensitivity and NPV and a lower misclassification rate. This simple and dependable test could have significant clinical implications in identifying patients who require monitoring for portal hypertension-associated complications and screening for HCC, particularly in middle and primary healthcare settings.

1. Introduction

Hepatitis B virus (HBV) infection remains a significant public health issue that causes severe morbidity and mortality. Worldwide, at least 2 billion individuals are infected with HBV, and over 650,000 people die each year because of chronic hepatitis B (CHB), significantly impacting the global health burden.[1–3] In China, the primary reason for the increased incidence of decompensated cirrhosis and hepatocellular carcinoma (HCC) is that patients with liver diseases do not seek medical attention until significant symptoms manifest, which occur when liver disease progresses to decompensated liver cirrhosis or even HCC.[4] Consequently, an early, and accurate diagnosis of cirrhosis, as well as the severity of liver fibrosis, has important clinical significance in determining the indications for antiviral therapy and selecting candidates who should be monitored for complications associated with portal hypertension and screened for HCC on a regular basis.[5]

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Liver biopsy is the gold standard for determining necroinflammatory activity and liver fibrosis. However, only a small portion of the liver is obtained during biopsy. As a result, this procedure might not detect the lesion characteristics in the whole liver due to heterogeneity in the distribution of lesions.[6] Furthermore, liver biopsy is an invasive and costly procedure that carries specific risks, including significant intraperitoneal hemorrhage.[7] Thus, liver biopsy is limited in its clinical application in the screening and follow-up of patients with CHB.

Noninvasive methods, including the aspartate aminotransferase (AST)-to-platelet ratio index (APRI),[8,9] fibrosis score based on four factors (FIB-4),[10,11] and liver stiffness (staged using 1-dimensional ultrasound transient elastography),[12,13] that reflect the dynamic process of the fibrotic burden have gained attention. However, due to the lack of dedicated devices and experienced operators in primary hospitals, transient elastography cannot be widely applied.

The red cell volume distribution width (RDW)-to-platelet ratio (RPR) was proposed in 2013 as a simple method that outperformed APRI and FIB-4 in assessing stages of liver fibrosis associated with CHB infection. More importantly, RPR is among the most easily available and reproducible laboratory tests in daily clinical practice.[14,15] However, the diagnostic and predictive performance of this method has yet to be demonstrated.[16]

Due to the significant rates of misclassification, using conventional cut-off values of APRI and FIB-4 to manage patients with a high risk of cirrhosis is not recommended.[17] Sonneveld *et al.*[17] identified and validated a new cut-off value for APRI ($\leq$0.45) and FIB-4 ($\leq$0.70), which performed satisfactorily in ruling out cirrhosis with a high diagnostic accuracy. Thus, additional research is needed to demonstrate whether these cut-off values can be applied to patients with low HBV DNA levels and consistently normal alanine transaminase (ALT) levels and whether RPR also has a cut-off value that can accurately exclude patients with cirrhosis.

The aim of this study was to investigate and compare the accuracy of APRI, FIB-4, and RPR in detecting significant fibrosis and cirrhosis. More importantly, the grid search method was applied to identify accepted cut-off values for ruling out cirrhosis.

2. Patients and methods

2.1. Ethical approval

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University (approval No. [2019] 02-530-01). Written informed consent from each participant was obtained prior to liver biopsy.

2.2. Patients

Between September 2005 and September 2020, 2544 consecutive patients with CHB (HBsAg positive >6 months) who underwent liver biopsy at the Third Affiliated Hospital of Sun Yat-sen University were included in this retrospective analysis. A liver biopsy was performed in all patients to assess the extent of necroinflammatory activity and fibrosis. The exclusion criteria were as follows: history of decompensated cirrhosis or HCC; hematopathy; malignant tumors other than HCC during this research period; coinfection with hepatitis C virus or human immunodeficiency virus; ALT level >10 times the upper limit of normal (ULN); other chronic liver diseases, including alcoholic liver diseases, autoimmune liver disease, and non-alcoholic fatty liver disease (either radiographic or histologic demonstration of more than 5% hepatic steatosis in the absence of excessive alcohol consumption); and insufficient data.

2.3. Serum markers

All routine laboratory tests, including standard complete blood counts and biochemical parameters, were performed within 1 week before liver biopsy. The calculations were formulated as follows:

$$\text{APRI} = \frac{AST(IU/L)/ULN}{Platelets\left(10^9/L\right)} \times 100$$

$$\text{FIB-4} = \frac{Age(years) \times AST(IU/L)}{Platelets\left(10^9/L\right) \times \sqrt{ALT(IU/L)}}$$

$$\text{RPR} = \frac{RDW(\%)}{Platelets\left(10^9/L\right)}$$

We used an AST level of 40 U/L as the ULN.

2.4. Histological assessment

Percutaneous liver biopsy was performed under ultrasound guidance using a 16-gauge disposable needle. The sample length was required to be at least 15 mm and included at least 6 portal tracts. Inflammation grade (G0–G4) and fibrosis stage (S0–S4) were estimated based on Scheuer's classification.[18] Under the pathological stage system, $\geq$S2 and S4 were defined as significant fibrosis and cirrhosis, respectively. All pathological sections were observed and evaluated blindly and independently by two experienced and specialized pathologists using an optical microscope to improve the accuracy of diagnosis. When the two pathologists did not agree, another pathologist was invited to make an assessment, and a consensus was reached after discussion.

2.5. Statistical analyses

SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA) and MedCalc version 20.110 software (MedCalc Software, Ostend, Belgium) were used for all statistical analyses. Patients were randomly separated into the derivation and validation datasets. Continuous variables are expressed as the mean ± standard deviation, and categorical data are expressed as counts and percentages. Student's *t*-test and the Chi-squared test were applied to compare variables that were significantly different between the validation and derivation cohorts. The correlation between the APRI, FIB-4, and RPR values, and fibrosis stage was evaluated by Spearman's rank correlation coefficient. The comparison of the area under the receiver operating characteristic curve (AUROC) was performed by the DeLong test. All *P*-values were two-sided, and *P*-values $\leq$0.05 were deemed statistically significant.

A grid search of cut-off points was used to determine the cut-off value for optimization, aiming to achieve a sensitivity of >90% (meaning that <10% of individuals with cirrhosis are misdiagnosed as not having cirrhosis) and a negative predictive value (NPV) of >95%.

3. Results

3.1. Baseline characteristics

Overall, 2544 patients with CHB were enrolled in this study. In accordance with the inclusion and exclusion criteria, 2182 patients were included and separated into the derivation and validation datasets in a 6:4 ratio (Fig. 1). The characteristics of the two cohorts

are summarized in Table 1. There were no significant differences between the derivation and validation groups in any of the variables at the time of liver biopsy ($P > 0.05$). Significant fibrosis ($S \geq 2$) and cirrhosis (S4) were observed in 55.2% (722/1309) and 53.6% (468/873), and 13.4% (175/1309) and 13.4% (117/873), of the patients in the derivation and validation datasets, respectively.

3.2. Association between APRI, FIB-4, and RPR scores, and fibrosis stage

Results for the mean, median and standard deviation for RPR, FIB-4, and APRI at each fibrosis stage are presented in Supplementary Table 1 and Fig. 2. Each mean value for RPR, FIB-4, and APRI

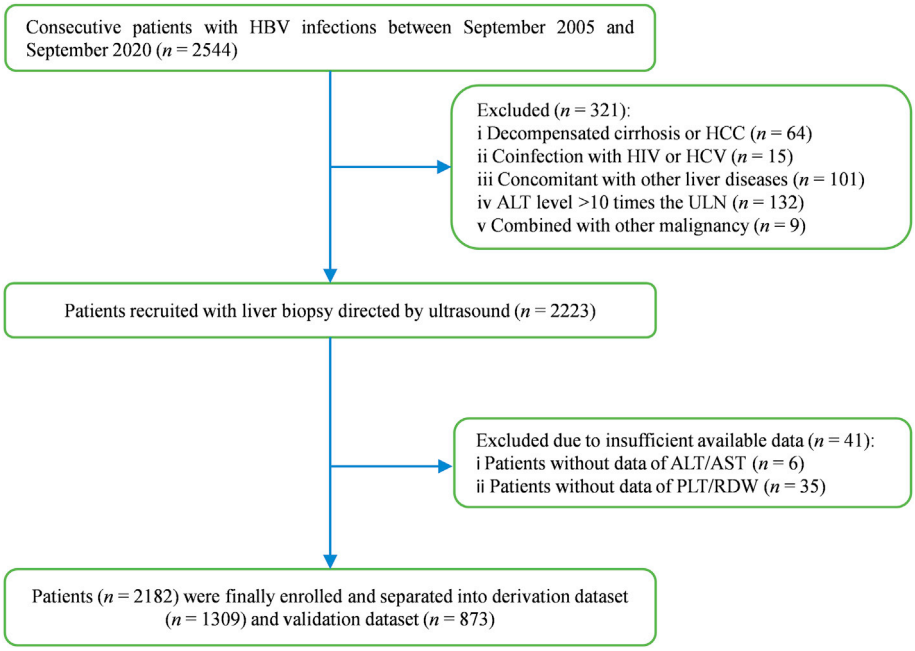

Fig. 1. Study flow for screening eligible patients. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PLT, platelet; RDW, red cell volume distribution width; ULN, upper limit of normal.

Table 1
Baseline characteristics of the study population ($N = 2182$).

Characteristic	Derivation dataset ($n = 1309$)	Validation dataset ($n = 873$)	*P*-value
Ages (years)	36.4 ± 9.2	39.9 ± 9.0	0.209
Male, *n* (%)	1026 (78.4)	674 (77.2)	0.517
WBC (10^9/L)	5.9 ± 1.8	5.9 ± 1.6	0.440
HGB (g/L)	143.8 ± 16.4	143.7 ± 16.6	0.808
PLT (10^9/L)	192.9 ± 57.5	194.3 ± 56.6	0.579
RDW (%)	13.2 ± 1.8	13.4 ± 3.8	0.268
MCV (fL)	86.7 ± 8.0	86.3 ± 8.9	0.342
ALT (U/L)	59.0 ± 54.4	58.3 ± 54.9	0.777
AST (U/L)	44.3 ± 37.0	43.7 ± 35.0	0.750
HBeAg positive, *n* (%)[a]	613 (46.8)	400 (45.8)	0.701
Detectable HBV DNA, *n* (%)[b]	228 (17.4)	156 (17.9)	0.821
APRI	0.437 (0.302–0.735)	0.427 (0.293–0.731)	0.628
FIB-4	0.978 (0.689–1.466)	0.955 (0.686–1.445)	0.593
RPR	0.765 ± 0.364	0.761 ± 0.352	0.795
Hypersplenism, *n* (%)	93 (7.1)	56 (6.4)	0.531
Inflammation stage, *n* (%)			0.227
G0–1	523 (40.0)	364 (41.7)	
G2	441 (33.7)	280 (32.1)	
G3	242 (18.5)	144 (16.5)	
G4	103 (7.9)	85 (9.7)	
Fibrosis stage, *n* (%)			0.595
S0–1	587 (44.8)	405 (46.4)	
S2	343 (26.2)	203 (23.3)	
S3	204 (15.6)	148 (17.0)	
S4	175 (13.4)	117 (13.4)	

Data were expressed as *n* (%), mean ± standard deviation, or median (25^{th} and 75^{th} percentiles).
Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis score based on four factors; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HGB, hemoglobin; MCV, mean corpuscular volume; PLT, platelet; RDW, red cell distribution width; RPR, red cell volume distribution width-to-platelet ratio; WBC, white blood cell.
[a] Data missing for 16 patients in the derivation dataset, 14 patients in the validation dataset.
[b] Data missing for 16 patients in the derivation dataset, 7 patients in the validation dataset.

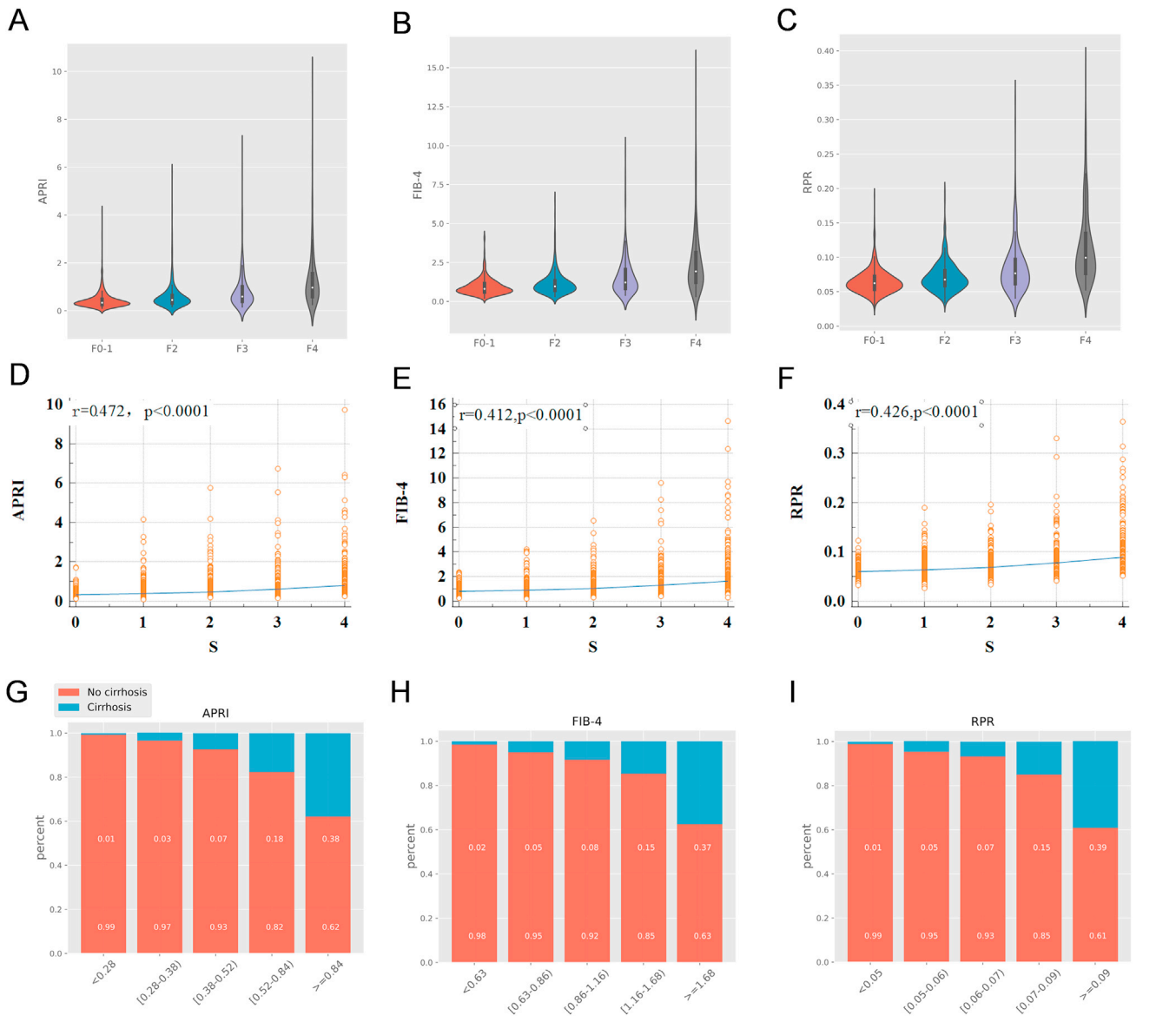

Fig. 2. Distribution of noninvasive markers of each fibrosis stage. Each mean value for APRI **(A)**, FIB-4 **(B)**, and RPR **(C)** increased in a stepwise pattern as fibrosis progressed. **Correlation with Scheuer's fibrosis stage in the derivation cohort.** Association between fibrosis stage and APRI **(D)**, FIB-4 **(E)**, and RPR **(F)**. Increases in APRI, FIB-4, and RPR scores were linked to Scheuer's fibrosis stage (all $P < 0.0001$). Relationship between APRI **(G)**, FIB-4 **(H)**, and RPR **(I)** with cirrhosis. Data were divided into quintiles. Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis score based on four factors; RPR, red cell volume distribution width-to-platelet ratio.

increased in a stepwise pattern as fibrosis increased (Fig. 2A–C). Increases in APRI, FIB-4, and RPR scores were linked to Scheuer's fibrosis stage. APRI had the highest correlation coefficient (r = 0.472) followed by RPR (r = 0.426) and FIB-4 (r = 0.412) (Fig. 2D–F). Higher APRI, FIB-4, and RPR scores are associated with the incidence of cirrhosis. For APRI, cirrhosis was detected in 3 (1%) patients in the lowest quintile and in 100 (38%) patients in the highest quintile. For FIB-4, the incidence of cirrhosis was 2% (5/262) in the lowest quintile and 37% (97/262) in the highest quintile. Similarly, for RPR, cirrhosis was detected in 3 (1%) patients in the lowest quintile and 102 (39%) patients in the highest quintile (Fig. 2G–I).

3.3. Comparisons of the diagnostic accuracy of APRI, FIB-4, and RPR

Receiver operating characteristic (ROC) curves and AUROCs of APRI, FIB-4, and RPR are shown in Table 2 and Fig. 3, respectively. Regarding the diagnostic performance of RPR, the AUROC was 0.699 for significant fibrosis (S2–S4) and 0.821 for cirrhosis (S4). For significant fibrosis, the performances of APRI, FIB-4, and RPR were less favorable. The AUROC value for RPR was not as high as that for APRI (0.731, $P = 0.0173$) but was equivalent to that for FIB-4 (0.694, $P = 0.7209$). Subgroup analyses results are shown in Supplementary Table 2. There was no statistically significant influence on the diagnostic accuracy of significant fibrosis in all subgroups. For cirrhosis, RPR was comparable to APRI (0.818, $P = 0.7905$) and FIB-4 (0.803, $P = 0.2395$). Based on the results of our subgroup analyses (Supplementary Table 2), the AUROC of APRI was substantially higher than that of the abnormal ALT group for cirrhosis (0.864 *vs.* 0.761; $P = 0.0025$). Furthermore, individuals who received antiviral therapy within 6 months performed better than those who were not treated (AUROC, 0.907 *vs.* 0.797; $P = 0.0002$). For FIB-4, the AUROC in patients with a normal corpuscular volume was lower than that in patients with microcytosis (0.788 *vs.* 0.882; $P = 0.0141$).

Table 2
Diagnostic performances of APRI, FIB-4, and RPR and their optimal cut-off values for the prediction of significant liver fibrosis and cirrhosis in the derivation cohort ($n = 1309$).

Fibrotic levels	Scores	AUROC (95%CI)	Cut-off values	Number identified (%)	Sen	Spe	PPV	NPV	NLR
Significant liver fibrosis (S2–S4)	APRI	0.731 (0.706–0.755)	<0.50[a]	874 (66.8)	0.555	0.762	0.741	0.582	0.58
			>1.50[a]		0.126	0.976	0.867	0.476	0.90
			0.46	–	0.625	0.724	0.736	0.611	0.52
	FIB-4	0.694 (0.669–0.719)	1.18	–	0.513	0.765	0.728	0.561	0.64
	RPR	0.699 (0.673–0.723)	0.0625[c]	–	0.699	0.545	0.654	0.596	0.55
			0.07	–	0.582	0.729	0.725	0.586	0.57
Cirrhosis (S4)	APRI	0.818 (0.796–0.838)	<1.00[a]	1161 (88.7)	0.497	0.892	0.414	0.920	0.56
			>2.00[a]		0.171	0.973	0.492	0.884	0.85
			≤0.45[b]	676 (51.6)	0.874	0.577	0.242	0.967	0.22
			0.53	–	0.811	0.679	0.281	0.959	0.28
	FIB-4	0.803 (0.781–0.825)	<1.45[d]	973 (74.3)	0.646	0.805	0.338	0.936	0.44
			≤0.70[b]	335 (25.6)	0.954	0.288	0.171	0.976	0.16
			1.30	–	0.714	0.748	0.304	0.944	0.38
	RPR	0.821 (0.799–0.842)	0.0685[c]	–	0.874	0.579	0.242	0.968	0.22
			0.08	–	0.703	0.782	0.332	0.945	0.38

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under the receiver operating characteristic curve; FIB-4, fibrosis score based on four factors; NLR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; RPR, red cell volume distribution width-to-platelet ratio; Sen, sensitivity; Spe, specificity.
[a] Cut-off values of APRI recommended by WHO.
[b] Cut-off values recommended by Sonneveld *et al.*[17] Used to rule out cirrhosis.
[c] Cut-off values of RPR recommended by Lee *et al.*[15]
[d] Conventional cut-off value of FIB-4 used to rule out advanced fibrosis.

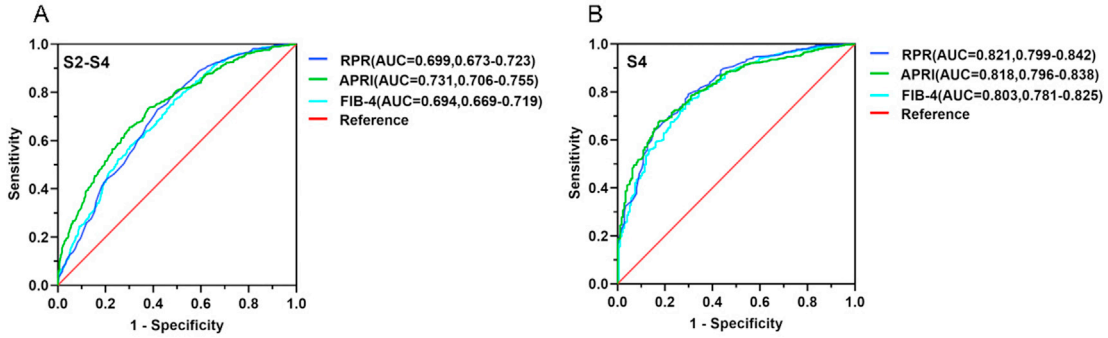

Fig. 3. ROC curves of RPR, APRI, and FIB-4. ROC curves of RPR, APRI, and FIB-4 in the prediction of significant fibrosis (S2–S4) **(A)** and cirrhosis (S4) **(B)** in the derivation cohort. Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; AUC, area under the curve; FIB-4, fibrosis score based on four factors; ROC, receiver operating characteristic; RPR, red cell volume distribution width-to-platelet ratio.

When the conventional cut-off values of S2–S4 were used, 33.2% (435/1309) of individuals had an APRI of 0.50–1.50 and hence were not classified (Table 2). Additionally, 41.6% (318/765) of the individuals with significant fibrosis had an APRI of <0.50 and were wrongly identified as having no significant fibrosis. Some individuals (23.9%, 140/587) without significant fibrosis had an APRI of >0.50 in the derivation cohort, and 49.1% (288/587) of those patients had an RPR of >0.0625.

For cirrhosis, 11.3% (148/1309) of the patients had an APRI of 1.00 (2.00 and hence were not classified when traditional cut-off values were applied. The positive predictive value (PPV) of an APRI of >2.00 was 49.2%, and the NPV of an APRI of <1.00 was 92.0%. In the derivation dataset, 50.3% (88/175) of the patients with cirrhosis had an APRI of <1.00 and were improperly classified as having no cirrhosis. As there are no commonly acknowledged cut-off values to diagnose cirrhosis with FIB-4, the maximum Youden index was used to calculate the cut-off value. An FIB-4 of 1.30 had a PPV of 0.304 and an NPV of 0.944. According to Lee *et al.*,[15] the PPV, and NPV were 24.2% and 96.8%, respectively, when using 0.0685 as the threshold for RPR (Table 2). Furthermore, 12.6% (22/175) of patients with cirrhosis had an RPR lower than the recommended cut-off value of 0.0685.

In the study, a high rate of incorrect classifications was observed when using conventional cut-off values or values computed by the maximum Youden index to estimate the severity of fibrosis. Consequently, these cut-offs may not be appropriate to guide clinicians in making perfect decisions. It is noteworthy that all cut-off values for these three classical models had a high NPV (>0.95) to predict the presence or absence of cirrhosis. Based on the cut-off values recommended by Sonneveld,[17] excluding patients with cirrhosis has a sensitivity of 0.954 and an NPV of 0.976 for FIB-4. However, only 25.6% of patients in our research population could be identified. Similarly, 51.6% of patients was identified by using an APRI of ≤0.45 with an NPV of 0.967 but an unsatisfactory sensitivity of 0.874 (Table 2). Moreover, no cut-off values for RPR have been proposed to exclude cirrhosis with an NPV of >0.95 and a sensitivity of >0.90. As a result, it is necessary to establish new cut-off values for these three noninvasive tests.

3.4. Identifying optimal cut-off values for APRI, FIB-4, and RPR to rule out cirrhosis in patients with CHB

To determine optimal cut-off values for ruling out cirrhosis, a grid search of cut-off points was performed. Fig. 4 shows the sensitivity and NPV observed in the derivation dataset. The performance of newly identified cut-off values in ruling out cirrhosis was unsatisfactory due to the low sensitivity, PPV, and identification rates. According to our preset criteria, the grid search identified

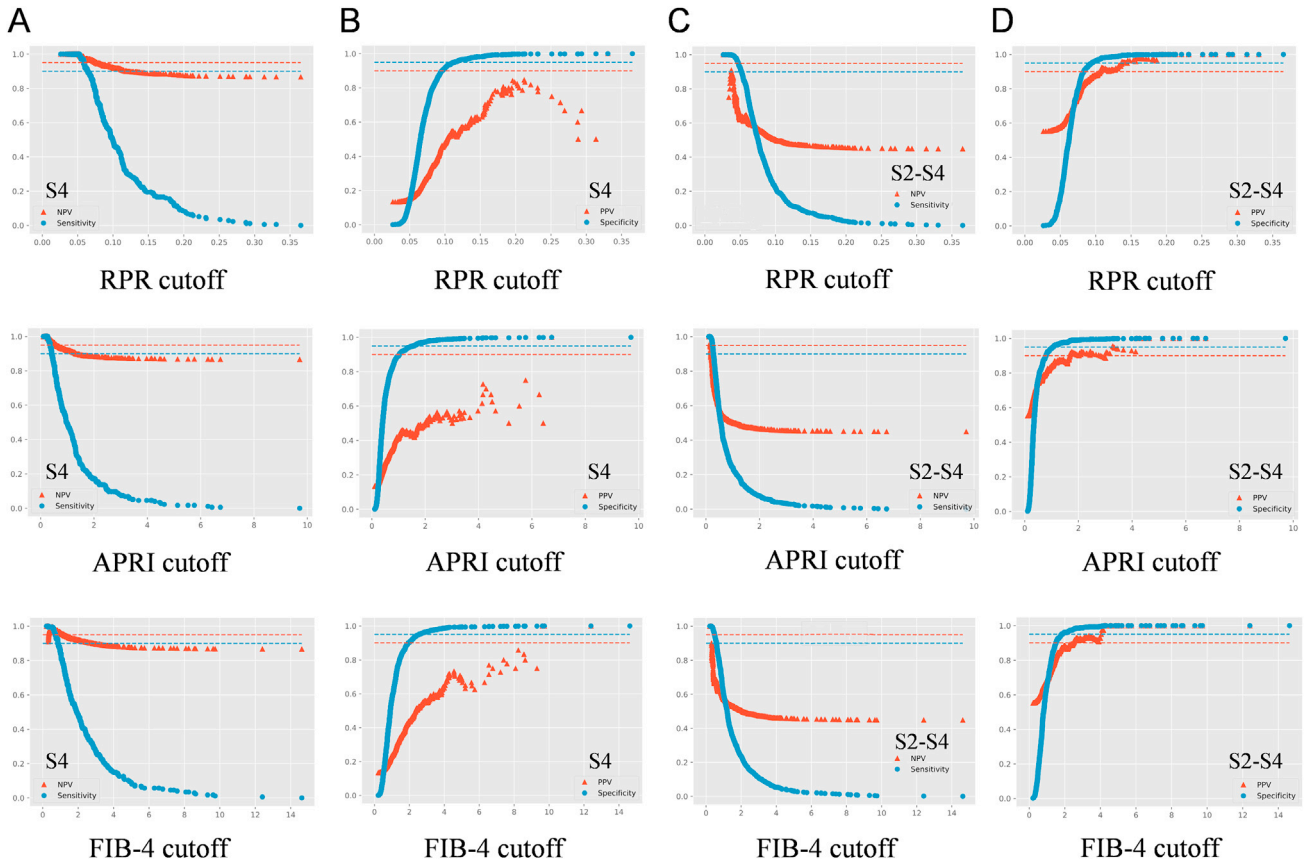

Fig. 4. Diagnostic performance for the prediction of cirrhosis (S4) and significant fibrosis (S2–S4) in the derivation dataset. NPV and sensitivity for various cut-off values of RPR, APRI, and FIB-4 **(A, C)**. PPV and specificity for various cut-off values of RPR, APRI, and FIB-4 **(B, D)**. Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, fibrosis score based on four factors; NPV, negative predictive value; PPV, positive predictive value; RPR, red cell volume distribution width-to-platelet ratio.

an APRI of 0.40, FIB-4 of 0.80, and RPR of 0.06 as ideal cut-off values to exclude cirrhosis. The overall performance of these cut-off values is summarized in Table 3. An RPR of ≤0.06 can exclude cirrhosis in 33.4% of patients (728/2182), and only 5.1% (15/292) of patients with cirrhosis were incorrectly classified as having no cirrhosis in the overall study population (Table 3). In the validation cohort, RPR had a sensitivity and NPV of 97.4% and 99.0%, respectively, and only misclassified 3 of 117 (2.6%) patients. According to the subgroup analysis, RPR had a relatively consistent performance in ruling out cirrhosis (Supplementary Table 3). In the derivation cohort, an FIB-4 of 0.80 had a sensitivity of 92.6%, an NPV of 97.2%, and misclassified 7.4% (13/175) of patients with cirrhosis. Moreover, an APRI of 0.40 had a sensitivity of 92.0%, an NPV of 97.6%, and misclassified 8.0% (14/175) of patients with cirrhosis (Table 3). Meanwhile, all new cut-off values established in this study, especially RPR, also performed well in the validation cohort. Subgroup analysis showed excellent NPVs and low rates of misclassification in all subgroups, except for patients with an ALT level below the ULN for APRI and those younger than 30 years for FIB-4 (Supplementary Table 3).

Although the primary objective of our study was to identify cut-off values for excluding cirrhosis, we also performed similar analyses for ruling in (a specificity of >95% and a PPV of >90%) and ruling out significant fibrosis (Fig. 4). Although we were unable to identify clinically useful cut-off values for ruling out significant fibrosis, we identified an APRI of ≥1.70, FIB-4 of ≥2.50, and RPR of ≥0.11 as useful for ruling in significant fibrosis (Supplementary Table 4). Unfortunately, only a limited number of patients were identified.

Table 3
Performance of newly identified cut-offs in the derivation cohort (n = 1309) and validation cohort (n = 873).

	Cut-off	Number identified, n (%)	Cirrhosis	Sen (%)	Spe (%)	NPV (%)	PPV (%)	DOR (%)	Misclassified, n (%)[a]
APRI									
Derivation	≤0.40	581 (44.4)	14	92.0	50.0	97.6	22.1	8.7	14 (8.0)
Validation	≤0.40	407 (46.6)	9	92.3	52.6	97.8	23.2	7.5	9 (7.7)
FIB-4									
Derivation	≤0.80	463 (35.4)	13	92.6	39.7	97.2	19.1	12.2	13 (7.4)
Validation	≤0.80	321 (36.8)	7	94.0	41.5	97.8	19.9	8.9	7 (6.0)
RPR									
Derivation	≤0.06	425 (32.5)	12	93.1	35.9	97.1	18.3	13.2	12 (6.9)
Validation	≤0.06	303 (34.7)	3	97.4	39.4	99.0	19.9	4.0	3 (2.6)

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; DOR, diagnostic odds ratio; FIB-4, fibrosis score based on four factors; NPV, negative predictive value; PPV, positive predictive value; RPR, red cell volume distribution width-to-platelet ratio; Sen, sensitivity; Spe, specificity.
[a] Patients with cirrhosis classified as having no cirrhosis.

4. Discussion

Changes in RDW and platelet counts are related to the development of chronic liver disease caused by hepatitis virus infection. Several pathophysiological mechanisms contribute to the rise in RDW: a long-term inflammatory response affects iron metabolism and the production of erythropoietin;[19,20] the decline in hepatic function will further affect the storage, metabolism, and synthesis of hematopoietic raw materials in the liver;[21] and hypersplenism among patients with liver cirrhosis accelerates the destruction of red blood cells. At the same time, in patients with cirrhosis, the metabolic, and synthetic functions of the liver are reduced, which affects the production of thrombopoietin, resulting in thrombocytopenia.[22] Additionally, platelets can secrete a variety of growth factors to promote liver regeneration, such as hepatocyte growth factor.[23,24] Therefore, compared with a single index, the combined index of RPR increased more significantly, which may have a better predictive effect. Several studies have shown that RPR may be an effective indicator for predicting significant fibrosis and cirrhosis especially in patients with CHB, autoimmune hepatitis, and primary biliary cirrhosis, whereas elevated RPR values are strongly linked to an increased risk of advanced fibrosis.[14,15,25,26]

Our study found that both RPR and FIB-4 had moderate diagnostic performance in identifying significant fibrosis, whereas APRI had a higher diagnostic value. For identifying cirrhosis, RPR was comparable to FIB-4 and APRI. For all three tests, the AUROC for diagnosing significant fibrosis was lower than that for diagnosing cirrhosis. The performance of APRI in identifying cirrhosis appears to be improved at normal ALT levels and in patients who receive antiviral therapy. In contrast, Liu *et al.*[27] reported that APRI seems to perform worse in detecting cirrhosis in patients who receive antiviral therapy. Moreover, this result contradicts the finding of Chen *et al.*,[28] who concluded that APRI seems to perform better in detecting cirrhosis when ALT levels are elevated.

For RPR, a cut-off value of 0.06 provided a high sensitivity (93.1%) and NPV (97.1%) in the derivation cohort. Compared to the traditional cut-off value (0.0685), the new cut-off value is mainly driven by a lower misclassification rate with a relatively higher NPV (0.967 *vs.* 0.937). Adjusting the cut-off value does increase the need for further testing (67.5% with the new RPR cut-off *vs.* 48.1% with the traditional cut-off). Meanwhile, we identified cut-off values for APRI of 0.40 and FIB-4 of 0.80. However, the sensitivity and NPV of RPR were generally higher than those of FIB-4 and APRI while having a lower rate of misclassification. Meanwhile, all new cut-off values established in this study, especially RPR, performed well in the validation cohort. More importantly, RPR had a more stable performance in all subgroups (Supplementary Table 3).

RPR has the advantage of relatively good compliance, affordability, and practicality. The complete blood count is one of the most used laboratory tests in daily clinical practice. Furthermore, the use of ALT levels alone to assess the extent of fibrosis is not perfect, and approximately one in five patients with CHB with an ALT value of $\leq$40 IU/L may have significant liver fibrosis.[29] With the abuse of anti-inflammatory and hepatoprotective drugs, some patients referred to tertiary care hospitals have already used hepatoprotective drugs, resulting in a decrease in aminotransferase levels. Based on this aspect, the RPR may be a better indicator of inflammation and fibrosis in patients with hepatitis B than APRI and FIB-4. Thus, it is envisioned that RPR will help in the early diagnosis and treatment of cirrhosis. To an extent, RPR can replace FIB-4 and APRI rather than supplement them. Moreover, in our subgroup analysis, an RPR of 0.06 was used to exclude cirrhosis that was not significantly affected by abnormal ALT levels and age, unlike APRI and FIB-4.

To our knowledge, this is the first study to determine RPR thresholds that can be used to exclude cirrhosis with a sensitivity >90% and an NPV >95%. Naturally, there are some limitations to this work. First, the single-center retrospective design may not be appropriate for generalization without external validation. However, the large sample size and low rate of missing data strengthen our conclusions. Second, although we used a similar incidence of cirrhosis as reported in other articles (12%–15% *vs.* 13.4%),[14,17] the calculation of the cut-off value could be affected by the prevalence of each stage of fibrosis, described as spectrum bias. Third, the optimal cut-off values for RPR, APRI, and FIB-4 can only be used to exclude cirrhosis; a more accurate and broad-coverage predictive model of the fibrosis stage is needed. Finally, one of the factors determining RPR is RDW, which is partially associated with patients with anemia, cardiovascular disease, and blood transfusions;[30] it is difficult for us to completely exclude these interference factors.

5. Conclusions

In conclusion, both RPR, and FIB-4 had moderate diagnostic value in identifying significant fibrosis, while APRI had a better performance. RPR was comparable to APRI and FIB-4 in the prediction of cirrhosis. A newly identified and validated cut-off value for RPR ($\leq$0.06) can be used to exclude more patients with cirrhosis than APRI and FIB-4 due to a higher sensitivity and NPV and a lower rate of misclassification. This easy and reliable test may have important clinical significance in selecting candidates who should be monitored for complications associated with portal hypertension and screened for HCC, especially in middle and primary hospitals.

Authors' contributions

Hongsheng Yu and Chao Li contributed equally to this work. Hongsheng Yu and Chao Li designed the study, performed the statistical analysis and drafted the manuscript. Mingkai Li, Abdukyamu Smayi, Bilan Yang, Kodjo-Kunale Abassa, Jianning Chen and Zixi Liang collected the clinical data and then reviewed the data. Bin Wu, Zixi Liang and Yidong Yang revised the manuscript. Yidong Yang and Bin Wu were responsible for the study conception, design, data analysis, and providing funding support. All authors have read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available on request from the corresponding authors.

Declaration of competing interest

Bin Wu is an editorial board member for *Liver Research* and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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Appendix A. Supplementary data

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