A novel noninvasive algorithm for the assessment of liver fibrosis in patients with chronic hepatitis B virus infection

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Summary
Several noninvasive blood biomarkers have been established for the assessment of liver fibrosis in patients with chronic hepatitis B virus (HBV) infection, but their clinical performance remains inconclusive. Here, we compared the diagnostic performance of these biomarkers and developed a novel algorithm for assessing liver fibrosis. Six hundred and sixteen chronically HBV-infected and treatment-naïve patients who underwent liver biopsy were enrolled and randomly divided into training (N=410) and internal validation cohorts (N=206). One hundred and fifty-nine patients from another centre were recruited as an external validation cohort. Receiver operating characteristic (ROC) curves were used to analyse the performance of the gamma-glutamyltransferase-to-platelet ratio (GPR), red cell volume distribution width-to-platelet ratio (RPR), FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI) and HBV DNA level against liver histology, and a novel algorithm was developed using the recursive partitioning and regression tree (RPART) method. In the training cohort, the area under the ROC curve of FIB-4 was significantly higher than that of APRI (P=.038) but was comparable to those of GPR, RPR and HBV DNA; however, the performance of the biomarkers was similar among the validation cohort. The established RPR-HBV DNA algorithm performed better in the training cohort than any individual blood biomarker, and the corresponding sensitivity, specificity, positive

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predictive value and negative predictive value were 63%, 90%, 72% and 80%, respectively. In the internal and external validation cohorts, the performance of the algorithm in assessing liver fibrosis was also superior to that of other biomarkers. These results suggest that the established RPR-HBV DNA algorithm might improve the diagnostic accuracy of liver fibrosis in treatment-naïve patients with chronic HBV infection, although additional studies are warranted to confirm these findings.

**KEYWORDS**

blood biomarkers, chronic hepatitis B virus infection, diagnosis, liver fibrosis

1 | INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a severe public health problem and is estimated to cause 686,000 deaths annually worldwide.1 In China, there are approximately 93 million chronic HBV carriers,2 accounting for approximately 38% of all HBV-infected patients worldwide.3 Overall, HBV causes approximately 45% of hepatocellular carcinoma (HCC) cases and 30% of cirrhosis cases.4 Maintaining viral suppression through antiviral therapy can reduce the occurrence of liver-related complications in these patients. In addition, the accurate assessment of liver fibrosis is crucial to identifying patients who are prone to severe clinical conditions and for making treatment decisions regarding patients with chronic HBV infection.5

Liver biopsy is currently recognized as the ‘gold standard’ for estimating liver fibrosis stage; however, this technique is invasive and confers an increased risk of complications.6 Sampling errors and inter- and intra-observer variability associated with liver biopsy may also make the assessment of liver fibrosis unreliable.7 Moreover, the dynamic surveillance of liver fibrosis using liver biopsy is limited and noninvasive methods are warranted. FibroScan is a noninvasive method that evaluates liver stiffness and has been reported to accurately reflect liver fibrosis in patients; however, its application in obese patients is limited, and the cost is relatively high.8-10 The analysis of blood biomarkers has been recognized as a simple, convenient and inexpensive noninvasive method for the assessment of liver fibrosis. Although several noninvasive models involving blood biomarkers have been developed, these models are far from optimal and have limited clinical value.11-19 Among these markers, the FIB-4 index17 and the aspartate aminotransferase-to-platelet ratio index (APRI)16,18,19 are widely used to assess patients with chronic hepatitis C (CHC), but their value for assessing patients who are chronically infected with HBV remains controversial.11,14,16

Recently, Lemoine et al. identified a novel simple index, the gamma-glutamyltransferase-to-platelet ratio (GPR), which exhibited better performance than FIB-4 and APRI in assessing liver fibrosis in chronically HBV-infected West African patients; however, this was not true for French populations.20 In contrast, Li et al. found that the performance of GPR was inferior to that of APRI for the assessment of liver fibrosis in Chinese populations.21 Another algorithm, the red cell volume distribution width-to-platelet ratio (RPR), was developed by Chen et al. and was found to be superior to APRI and FIB-4 for predicting significant fibrosis and cirrhosis.22 The quantitation of hepatitis B surface antigen (HBsAg) and HBV DNA level may be useful for the diagnosis of liver fibrosis in chronically HBV-infected patients.23-28 Several clinical studies have compared the utilities of these blood biomarkers for the diagnosis of liver fibrosis, but inconclusive results have been reported.12-14,29

The aims of this study were to systematically compare the diagnostic performance of established blood biomarkers and to establish an improved algorithm for the assessment of liver fibrosis in treatment-naïve chronically HBV-infected Chinese patients.

2 | MATERIALS AND METHODS

2.1 | Patients

For this study, 1,302 consecutive chronically HBV-infected patients, who underwent liver biopsy at Ruijin Hospital (Shanghai, China) from 2008 to 2015, were retrospectively recruited. Chronic HBV infection was defined as having blood that tested positive for HBsAg for at least 6 months. Patients were excluded from this study for the following reasons: excessive alcohol intake (n=46), co-infection with HIV or hepatitis C virus (HCV), a history of other chronic liver diseases (n=67), previous antiviral treatment history (n=214), hepatocellular carcinoma or other types of cancer (n=3) and the lack of sufficient biopsy samples or data pertaining to red cell volume distribution width (RDW), platelet count (PLT), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (n=356). In total, 616 eligible patients were included in this study and were randomly divided into a training cohort (N=410) and an internal validation cohort (N=216). All subjects provided written informed consent, and the study was performed according to the ethical guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Ruijin Hospital.

2.2 | External validation cohort

One external validation set of treatment-naïve HBV-infected patients (N=159) who attended Shanghai Public Health Clinical Centre between September 2014 and January 2016 were recruited to assess the diagnostic performance of the novel algorithm and other noninvasive biomarkers. The inclusion and exclusion criteria used were the same as those used for the patients who were enrolled at Ruijin Hospital. The patients provided written consent, and the study was performed according to the Declaration of Helsinki and approved by the Ethics Committee of Shanghai Public Health Clinical Centre.
2.3 | Liver histology evaluation

Liver biopsies were performed using 16-G biopsy needles under the guidance of ultrasound. The biopsy specimens were fixed with 10% formalin and embedded in paraffin, and routine staining was performed, including haematoxylin and eosin, Masson’s trichrome and reticular fibre staining. Liver fibrosis stage was assessed according to the METAVIR scoring system as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Significant fibrosis was defined as fibrosis stage ≥F2. All biopsy samples were reviewed by two liver pathologists. When the pathologists differed in their assessments, an agreement was reached by discussion.

2.4 | Laboratory tests

All blood samples were obtained, and laboratory variables were measured at the time of biopsy based on the manufacturer’s instructions. Blood biochemical parameters included ALT, AST and GGT. Haematological parameters included RDW and PLT. Virological parameters included HBV serological markers and HBV DNA. HBV serological markers were detected using a chemiluminescent microparticle enzyme immunoassay (CMIA, Abbott, Chicago, IL, USA). HBV DNA level was measured using real-time PCR (PJ Co. Ltd., Shenzhen, China) with a lower limit of detection of 500 IU mL⁻¹.

2.5 | Model calculations

GPR, RPR, FIB-4 and APRI were calculated as previously described: GPR=[GGT/ULN]/PLT(10⁹ L⁻¹)×100; RPR=RDW (%) PLT (10⁹ L⁻¹); FIB-4 = age (years)×AST [U L⁻¹]/PLT (10⁹ L⁻¹)×ALT [U L⁻¹]¹/²; and APRI = AST ([ULN])×100/PLT (10⁹ L⁻¹).

2.6 | Statistical analysis

Continuous variables are presented as median (interquartile range) and were compared using the Mann-Whitney U test. Categorical variables were compared using Chi-square test. Spearman’s rank correlations were performed to evaluate the biomarkers for assessing fibrosis stage. The HBV DNA level was logarithmically transformed. The diagnostic performance of the blood biomarkers was compared by calculating the area under the ROC curves (AUC), and the De Long method was applied using Medcalc software. Optimal cut-off values were obtained from the Youden’s index of the training cohort and were further confirmed using the internal and external validation cohorts. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at the optimal cut-off value for each biomarker. We applied the recursive partitioning and regression tree (RPART) method to establish a novel algorithm for the diagnosis of liver fibrosis based on the basic characteristics and blood biomarkers of the patients. The RPART programme employed 10-fold cross-validation procedures to identify the optimal tuning complexity parameter (CP) value for the training cohort. The best classification model was defined as the least complex model with a 10-fold cross-validation error not exceeding one standard error above the minimum cross-validation error. The proportions of the correctly classified patients according to the algorithm and according to the other biomarkers were compared using the McNemar test. All analyses were performed using the SPSS statistical package, version 16.0 (SPSS, Chicago, IL, USA) or the RPART package of R, version 3.0 (http://www.r-project.org/). A two-sided P value of less than .05 was considered statistically significant.

3 | RESULTS

3.1 | Training cohort

3.1.1 | Baseline characteristics of patients in the training cohort

The flow chart for patient enrolment is shown in Fig. S1. To assess and validate the diagnostic ability of noninvasive biomarkers, 616 patients from Ruijin Hospital were randomly assigned to a training cohort (N=410) or to an internal validation cohort (N=206). Similar baseline characteristics, hepatic fibrosis distribution and laboratory parameters were identified between the two cohorts (Table 1). The median age of the 410 patients in the training cohort (277 men and 133 women) was 37 years. The median GPR, RPR, FIB-4 and APRI were 0.22, 0.07, 1.15 and 0.53, respectively (Table 1). The distribution of each fibrosis stage in the training cohort was as follows: F0, 85 (20.70%) patients; F1, 175 (42.70%) patients; F2, 65 (15.90%) patients; F3, 40 (9.70%) patients; and F4, 45 (11.00%) patients (Table 1).

3.1.2 | Correlation between blood biomarkers and the liver fibrosis stage

Figure 1 presents the levels of GPR, RPR, FIB-4, APRI and HBV DNA in the training cohort according to liver fibrosis stage as measured by the METAVIR scoring system. Fibrosis stage was positively correlated with GPR (Spearman’s r=.399, P<.0001), RPR (Spearman’s r=.331, P<.0001), FIB-4 (Spearman’s r=.385, P<.0001) and APRI (Spearman’s r=.337, P<.0001) but was inversely correlated with HBV DNA (Spearman’s r=−.229, P<.0001). We performed ROC analysis to evaluate the diagnostic performance of the blood biomarkers (Figure 2A). In the training cohort, the AUC of FIB-4 was 0.712 (95% CI = 0.665-0.755) and was significantly higher than that of APRI (AUC=.661, 95% CI = 0.612-0.706; P=.038) and comparable to those of HBV DNA (AUC=.692, 95% CI = 0.645-0.736; P=.586), GPR (AUC=.700, 95% CI = 0.654-0.744; P=.700) and RPR (AUC=.706, 95% CI = 0.659-0.750; P=.815).

3.1.3 | Construction of a novel assessment algorithm for significant liver fibrosis

In the training cohort, an optimal GPR cut-off value of 0.1581 generated a sensitivity of 83%, a specificity of 45%, a PPV of 47% and an...
FIGURE 1  Box plots of blood gamma-glutamyltranspeptidase-to-platelet ratio (GPR), red cell volume distribution width-to-platelet ratio (RPR), FIB-4 index, aspartate aminotransferase-to-platelet ratio index (APRI) and HBV DNA according to fibrosis stage in the training cohort. The top and bottom of the whiskers represent the minimum and maximum values, respectively. The top and bottom of the boxes represent the first and third quartiles, respectively, and the horizontal lines across the boxes represent the median values. Correlation between the different stages of fibrosis was calculated using the Kruskal-Wallis test or the Mann–Whitney U test.

TABLE 1  Baseline characteristics of patients in the training and validation (internal and validation) cohorts

|                          | Training (n=410) | Internal validation (n=206) | $P$ value* | External validation (n=159) | $P$ value** |
|--------------------------|-----------------|-----------------------------|------------|-----------------------------|-------------|
| Male, n (%)              | 277 (67.6)      | 136 (66)                    | .701       | 106 (66.7)                  | .838        |
| Age (y)                  | 37.00 (31.00-47.00) | 37.00 (31.00-46.00)         | .933       | 36.00 (29.50-44.00)         | .195        |
| ALT (IU L$^{-1}$)        | 43.00 (29.00-74.00) | 40.00 (27.25-71.75)         | .345       | 48.00 (29.00-80.50)         | .717        |
| AST (IU L$^{-1}$)        | 34.00 (26.00-49.75) | 32.00 (24.00-46.00)         | .133       | 35.00 (25.00-56.00)         | .888        |
| GGT (IU L$^{-1}$)        | 24.00 (16.00-37.75) | 24.00 (16.00-36.00)         | .878       | 31.00 (17.50-57.50)         | <.001       |
| RDW (%)                  | 12.80 (12.30-13.40) | 12.80 (12.20-13.30)         | .578       | 12.80 (12.10-13.30)         | .853        |
| PLT (10$^9$ L$^{-1}$)    | 171.0 (140.0-215.0) | 173.00 (142.00-209.00)      | .918       | 155.00 (109.00-183.00)      | <.001       |
| HBV DNA (log$_{10}$ IU mL$^{-1}$) | 6.3 (4.64-7.88) | 6.14 (4.62-7.69) | .359       | 6.06 (4.19-7.70) | .198 |
| HBeAg positive, n (%)    | 231 (63.60)     | 121 (65.10)                 | .743       | 104 (65.40)                 | .697        |
| GPR                      | 0.22 (0.13-0.38) | 0.22 (0.14-0.39)            | .882       | 0.34 (0.20-0.77)            | <.001       |
| RPR                      | 0.07 (0.06-0.09) | 0.07 (0.06-0.09)            | .951       | 0.08 (0.07-0.12)            | <.001       |
| FIB-4                    | 1.15 (0.82-1.79) | 1.18 (0.80-1.67)            | .506       | 1.30 (0.90-1.99)            | .022        |
| APRI                     | 0.53 (0.35-0.85) | 0.48 (0.33-0.76)            | .158       | 0.68 (0.37-1.11)            | .007        |
| Inflammation Stage (n, %)| 70 (17.1)/237 (57.8)/73 (17.8)/30 (7.3) | 38 (18.4)/113 (54.9)/37 (18.0)/18 (8.7) | .870       | 25 (15.70)/76 (47.80)/28 (17.60)/30 (18.90) | <.001 |
| Fibrosis Stage (n, %)    | 85 (20.7)/175 (42.7)/65 (15.9)/40 (9.7)/45 (11.0) | 47 (22.8)/101 (49.0)/19 (9.2)/14 (6.8)/25 (12.2) | .113       | 16 (10.10)/72 (45.30)/27 (17.00)/19 (11.90)/25 (15.70) | .036 |

* $P$ value for the comparison between the training and internal validation sets.
** $P$ value for the comparison between the training and external validation sets.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; RDW, red cell volume distribution width; PLT, platelet count; HBBeAg, hepatitis B e antigen; GPR, GGT-to-PLT; RPR, RDW-to-PLT; FIB-4, fibrosis index based on the four factors; APRI, AST-to-PLT ratio index.
NPV of 83% according to Youden’s index. The optimal cut-off value of RPR was 0.0804, and the corresponding sensitivity, specificity, PPV and NPV were 63%, 72%, 57% and 77%, respectively. The optimal cut-off value of FIB-4 was 1.1951, and the corresponding sensitivity, specificity, PPV and NPV were 69%, 66%, 54% and 79%, respectively. The optimal cut-off value of API4 was 0.4135, and the corresponding sensitivity, specificity, PPV and NPV were 84%, 46%, 47% and 83%, respectively. The optimal cut-off value of the HBV DNA level was 7.1, and the corresponding sensitivity, specificity, PPV and NPV were 89%, 54%, 53% and 90%, respectively (Table 2).

Using the RPART method, we established a novel assessment algorithm with 10-fold cross-validation in the training cohort (Figure 3). The combination of HBV DNA level and RPR was found to have the optimal diagnostic value for liver fibrosis in chronically HBV-infected patients. The corresponding sensitivity, specificity, PPV and NPV of the algorithm were 63%, 90%, 72% and 80%, respectively (Table 2). In comparison with previously developed blood biomarkers, the novel algorithm correctly identified 317 cases (77%) of 410 cases, better than the use of any previously developed blood biomarkers alone (P<.001) (Table 2).

3.2 | Internal validation cohort

In the internal validation cohort (n=206), 47 (22.80%) cases were F0, 101 (49.00%) cases were F1, 19 (9.20%) were F2, 14 (6.80%) cases...
were F3, and 25 (12.2%) cases were F4 based on the liver histology results (Table 1). Regarding diagnostic performance, the AUC (95% CI) was 0.605 (0.534-0.672) for APRI; 0.647 (0.578-0.712) for FIB-4; 0.673 (0.605-0.737) for GPR; 0.676 (0.607-0.739) for RPR; and 0.688 (0.619-0.750) for HBV DNA (Figure 2B). No significant difference was noted for the AUCs among these blood biomarkers in the internal validation cohort (P > 0.05).

Using the optimal cut-off values derived from the training cohort, the corresponding sensitivity for the novel algorithm was 53%; the specificity was 76%; the PPV was 47%; and the NPV was 87%. The algorithm correctly identified 144 cases (70%) of the total 206 cases in the internal validation cohort, a significantly higher number than those for GPR (P = 0.001), RPR (P < 0.001), FIB-4 (P = 0.001), APRI (P < 0.001) and HBV DNA (P = 0.004) (Table 2).

3.3 | External validation cohort

The median GGT and PLT were significantly different between the external validation cohort (N=159) and the training cohort. The median GPR, RPR, FIB-4 and APRI were 0.34, 0.08, 1.30 and 0.68, respectively. The distribution of each fibrosis stage was as follows: F0, 16 (10.10%) patients; F1, 72 (45.30%) patients; F2, 27 (17.00%) patients; F3, 19 (11.90%) patients; and F4, 25 (15.70%) patients (Table 1). For predicting significant fibrosis, the AUC (95% CI) for GPR, FIB-4, RPR, and APRI was 0.807 (0.737-0.865), 0.776 (0.704-0.839), 0.777 (0.704-0.839) and 0.753 (0.679-0.818), respectively. No significant difference was noted for the AUCs among these blood biomarkers (P > 0.05). The distribution of the blood biomarkers according to liver fibrosis stage is shown in Fig. S2B.

The performance of the blood biomarkers using the optimal cut-off values derived from the training cohort is shown in Table 2. Using the optimal cut-off values derived from the training cohort, the corresponding sensitivity for the novel RPR-HBV DNA algorithm was 68%; the specificity was 81%; the PPV was 74%; and the NPV was 76%. The algorithm correctly identified 119 cases (75%) of the total 159 cases in the external validation cohort. GPR and HBV DNA correctly identified 96 cases (75%) and 100 cases (63%) out of the total 159 cases, respectively, significantly lower numbers than those obtained

| TABLE 2 | Diagnostic performance of the studied blood biomarkers and the established RPR-HBV DNA algorithm for diagnosing significant liver fibrosis in treatment-naïve and chronically hepatitis B virus-infected patients |
| GPR | RPR | FIB-4 | APRI | HBV DNA (log_{10} IU mL^{-1}) | RPR-HBV DNA algorithm |
| --- | --- | --- | --- | --- | --- |
| **Training cohort** | | | | | |
| Optimal cut-off | 0.1581 | 0.0804 | 1.1951 | 0.4135 | 7.1 |
| Sensitivity (%) | 83 | 63 | 69 | 84 | 89 | 63 |
| Specificity (%) | 45 | 72 | 66 | 46 | 54 | 90 |
| Positive predictive value (%) | 47 | 57 | 54 | 47 | 53 | 72 |
| Negative predictive value (%) | 83 | 77 | 79 | 83 | 90 | 80 |
| No. biopsy correctly avoided (n, %) | 242 (59) | 282 (69) | 274 (69) | 246 (60) | 273 (67) | 317 (77) |
| No. incorrect diagnosis (n, %) | 168 (41) | 128 (31) | 136 (31) | 164 (40) | 137 (33) | 93 (23) |

| **Internal validation cohort** | | | | | |
| Sensitivity (%) | 83 | 53 | 62 | 71 | 93 | 53 |
| Specificity (%) | 43 | 67 | 56 | 43 | 44 | 76 |
| Positive predictive value (%) | 36 | 39 | 36 | 33 | 39 | 47 |
| Negative predictive value (%) | 86 | 79 | 79 | 78 | 94 | 87 |
| No. biopsy correctly avoided (n, %) | 111 (54) | 130 (63) | 119 (58) | 105 (51) | 119 (58) | 144 (70) |
| No. incorrect diagnosis (n, %) | 95 (46) | 76 (37) | 87 (42) | 101 (49) | 87 (42) | 62 (30) |

| **External validation cohort** | | | | | |
| Sensitivity (%) | 96 | 82 | 74 | 90 | 75 | 68 |
| Specificity (%) | 32 | 58 | 61 | 47 | 53 | 81 |
| Positive predictive value (%) | 53 | 61 | 60 | 58 | 56 | 74 |
| Negative predictive value (%) | 90 | 80 | 75 | 85 | 72 | 76 |
| No. biopsy correctly avoided (n, %) | 96 (60) | 109 (69) | 107 (67) | 105 (66) | 100 (63) | 119 (75) |
| No. incorrect diagnosis (n, %) | 63 (40) | 50 (31) | 52 (33) | 54 (34) | 59 (37) | 40 (25) |

*The diagnostic ability of the RPR-HBV DNA algorithm is significantly different from those of GPR and HBV DNA.*
using the algorithm ($P=0.009$ for GPR and $P=0.001$ for HBV DNA). RPR, FIB-4 and APRI correctly identified 109 cases (69%), 107 cases (67%) and 105 cases (66%) out of the total of 159 cases, respectively; these values were lower than those obtained using the algorithm, but the differences were not significant ($P=0.099$ for RPR, $P=0.104$ for FIB-4 and $P=0.087$ for APRI; Table 2).

4 | DISCUSSION

Early detection of significant fibrosis is essential for reaching antiviral therapy decisions in chronically HBV-infected patients. The present study compared the diagnostic value of previously established blood biomarkers including GPR, RPR, FIB-4 and APRI for assessing liver fibrosis in a large population of Chinese patients with chronic HBV infection and established a novel algorithm for the assessment of liver fibrosis that yielded better performance than previous methods. Considering the limitations of liver biopsy and the relatively high cost of the FibroScan, noninvasive methods to identify significant fibrosis in chronically HBV-infected patients are urgently needed in clinical practice. The novel RPR-HBV DNA algorithm presented here may be helpful in monitoring significant fibrosis in treatment-naïve chronically HBV-infected patients.

The blood biomarkers APRI and FIB-4 were first developed by Wai et al. and Sterling et al. and were found to be useful for the diagnosis of liver fibrosis in patients with CHC and in patients co-infected with HIV/HCV, respectively. WHO guidelines recommend the use of APRI for estimating liver fibrosis in patients with chronic HBV infection in resource-limited regions; however, these biomarkers exhibit lower sensitivity and specificity in clinical use, and their diagnostic value is limited and controversial for chronically HBV-infected patients. In our study, the performance of FIB-4 was slightly superior to that of APRI in the training cohort, but the diagnostic value of these markers was comparable in the internal and external validation cohorts. The discrepancy may be due to the small sample size of the validation cohort and the different basic characteristics of the included patients.

Lemoine et al. developed a noninvasive biomarker, GPR, for diagnosing liver fibrosis in chronically HBV-infected patients. The authors found that GPR was superior to APRI and FIB-4 in two African cohorts, but the diagnostic performance of these biomarkers were similar in a French cohort. Li et al. found that GPR was inferior to APRI but comparable to FIB-4 in Chinese patients chronically infected with HBV. However, when restricted to patients with high HBV DNA levels and normal or mildly elevated ALT levels, GPR performed better than APRI and FIB-4 in the assessment of liver fibrosis. In the current study, we found that the performance of GPR was comparable to that of APRI and FIB-4 among patients with chronic HBV infection in both the training and validation sets. Previous studies showed that for evaluating significant fibrosis, the AUC of GPR ranges from 0.66 to 0.80. Using the optimal cut-off value, the corresponding sensitivity ranged from 49% to 83%, and the specificity ranged from 43% to 83%. The difference in the diagnostic ability of GPR between these studies may be caused by differences in the basic characteristics and spectrum bias in the fibrosis distribution of these patients. The GPR index was generated based on GGT and PLT. GGT can be affected by biliary tract disease and by some types of drugs, and this had not been evaluated in the reported studies. Thus, the value of GPR for the assessment of liver fibrosis in patients with chronic HBV infection needs to be determined using further studies, and other clinical factors that may affect the GPR level should be identified.

Chen et al. reported the use of another noninvasive biomarker, RPR, to evaluate liver fibrosis in Chinese chronically HBV-infected patients. The authors concluded that RPR is a more powerful
diagnostic index for significant fibrosis than APRI and FIB-4. Lee et al. reported that RPR yielded comparable results to APRI and FIB-4 but was inferior to transient elastography (TE) for assessing significant fibrosis in a Korean population with chronic HBV infection.34 In our study, we found that RPR yielded similar results to GPR, FIB-4 and APRI for the assessment of liver fibrosis in patients with chronic HBV infection, although this finding requires further validation.

The natural history of chronic HBV infection is different and more complex than that of HCV infection.25,27,28,36,46,47 Serum HBV DNA level, which is commonly used during the treatment of chronically HBV-infected patients, has crucial implications for the natural history of chronic HBV infection. To date, several studies have identified a significant inverse correlation between HBV DNA level and liver fibrosis stage.23,27,28,48,49 Xie et al. reported that the HBV DNA level is significantly decreased during the process of liver fibrosis.33 Serum HBV DNA levels were lower in chronically HBV-infected and treatment-naïve patients with fibrosis compared to the levels in those without fibrosis and this marker may have diagnostic value for evaluating liver fibrosis in treatment-naïve patients.23,27 With the ROC method, we found that HBV DNA levels had comparable diagnostic value for liver fibrosis compared to FIB-4, APRI, RPR and GPR in the training and internal validation cohorts. When using the optimal cut-off, the NPV for HBV DNA level was high (90%, 94% and 72% in the training, internal validation and external validation cohorts, respectively); however, the PPV was low (53%, 39% and 56% in the training, internal validation and external validation cohorts, respectively). Using the RPART method, we established a novel RPR-HBV DNA algorithm that provided better diagnostic performance for liver fibrosis in the training cohort than any individual blood biomarker. Using the algorithm, 77% of patients were correctly identified. Importantly, the novel algorithm consisted of conventional blood biomarkers and the results of virological testing, which are routinely obtained in the management of chronically HBV-infected patients; no extra tests that would increase the medical burden on the patients are required. However, although the algorithm achieved high specificity and NPV, these came at the cost of sensitivity and PPV. In the external validation cohort, even though no significant difference existed between the results obtained using the algorithm and those obtained using individual biomarkers, the number of patients who were correctly diagnosed using the algorithm was higher than that obtained using individual biomarkers; this result might be due to differences in the basic characteristics of the patients and the small sample size of the external validation cohort. The FibroScan has been used to evaluate liver fibrosis in chronically HBV-infected patients based on a determination of liver stiffness; however, the FibroScan is relatively expensive, and specialized technicians are needed. This test is unavailable in less developed countries and regions. In the current study, we did not compare the performance of the algorithm and FibroScan due to the lack of FibroScan data for the recruited patients. A comparison between the algorithm and FibroScan using well-designed studies is warranted.

Several limitations should be acknowledged. First, this study was retrospective and used data obtained from two clinical centres. The results should be validated in prospective multicentre studies using larger sample sizes. Second, the distribution of fibrosis stage in chronically HBV-infected patients may differ between clinical studies, and the diagnostic performance of conventional blood biomarkers may be influenced by patient characteristics. Third, other clinical confounding factors such as quantitative HBsAg levels and HBV genotype may affect the liver fibrosis caused by HBV, and these factors should be considered in future studies.

In conclusion, we compared the use of conventional blood biomarkers, including GPR, RPR, FIB-4 and APRI, for evaluating liver fibrosis in Chinese patients with chronic HBV infection and established a novel algorithm for the assessment of liver fibrosis. More studies are warranted to confirm the diagnostic value of the algorithm for treatment-naïve chronically HBV-infected patients.

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AUTHORS’ CONTRIBUTION

Xin-Xin Zhang conceived and designed the experiments; Qi-Ming Gong, Dong-Hua Zhang and Yan Zhang performed the research; Ming-Yu Zhu, Pei-Zhan Chen and Xia Zou analysed the data; Zhi-Tao Yang, Jie Chen, Dao Huang and Xin-Xin Zhang contributed reagents/materials/analysis tools; Dao Huang and Xin-xin Zhang; Qiang Li and Liang Chen provided the external validation cohort; Ming-Yu Zhu, Pei-Zhan Chen and Xin-xin Zhang wrote the manuscript.

CONFLICTS OF INTEREST

The authors declared that they have no competing financial interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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