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Retrospective Study

Novel index for the prediction of significant liver fibrosis and cirrhosis in chronic hepatitis B patients in China

Liao MJ *et al*. AGPR predicting liver fibrosis and cirrhosis

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Abstract

BACKGROUND

Noninvasive, practical, and convenient means of detection for the prediction of liver fibrosis and cirrhosis in China are greatly needed.

AIM

To develop a precise noninvasive test to stage liver fibrosis and cirrhosis.

METHODS

With liver biopsy as the gold standard, we established a new index, [alkaline phosphatase (U/L) + gamma-glutamyl transpeptidase (U/L)/platelet (10^9/L) (AGPR)], to predict liver fibrosis and cirrhosis. In addition, we compared the area under the receiver operating characteristic curve (AUROC) of AGPR, gamma-glutamyl transpeptidase to platelet ratio, aspartate transaminase to platelet ratio index, and FIB-4 and evaluated the accuracy of these routine laboratory indices in predicting liver fibrosis and cirrhosis.

RESULTS

Correlation analysis revealed a significant positive correlation between AGPR and liver fibrosis stage ($P < 0.001$). In the training cohort, the AUROC of AGPR was 0.83 (95%CI: 0.78-0.87) for predicting fibrosis (≥ F2), 0.84 (95%CI: 0.79-0.88) for predicting extensive fibrosis (≥ F3), and 0.87 (95%CI: 0.83-0.91) for predicting cirrhosis (F4). In the validation cohort, the AUROCs of AGPR to predict ≥ F2, ≥ F3 and F4 were 0.83 (95%CI: 0.77-0.88), 0.83 (95%CI: 0.77-0.89), and 0.84 (95%CI: 0.78-0.89), respectively.

CONCLUSION

The AGPR index should become a new, simple, accurate, and noninvasive marker to predict liver fibrosis and cirrhosis in chronic hepatitis B patients.

Key Words: Liver; Fibrosis; Cirrhosis; Prediction; Novel noninvasive marker; Chronic hepatitis B

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Core Tip: Chronic hepatitis B virus (HBV) infection is highly endemic in China, and routine assessment of chronic hepatitis B patients is greatly needed to guide management and indicate the need for treatment. In this study, we established a new index to stage liver fibrosis and cirrhosis in patients with chronic HBV infection in China. In addition, the study compared the predictive performance between the new index and other noninvasive indices. The new index is suitable for regular monitoring and is crucial for the management of patients with liver fibrosis/cirrhosis.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a public issue that affects human health. Patients who are chronically infected with HBV have a tendency to develop liver fibrosis, liver cirrhosis and even more serious conditions, such as hepatocellular carcinoma (HCC). It has been reported that liver fibrosis can be reversed in patients with varying degrees of fibrosis by removing pathogenic factors, including HBV infection and alcohol[1,2]. It would benefit patients to obtain early diagnosis and effective treatment of liver fibrosis before the disease worsens. Hence, measures should be taken to achieve early diagnosis of liver fibrosis to avoid disease progression caused by HBV infection.

Liver biopsy has always been regarded as the gold standard to evaluate liver histology and assess the degree of fibrosis[3]. However, liver biopsy has shortcomings. Like any surgery, liver biopsy carries some risks, such as puncture of the lung or gallbladder, infection, bleeding, and pain, although these complications are rare[4]. In

addition, a major problem is the sampling error and significant variability of fibrosis assessment by liver biopsy[5]. Transient elastography performed with FibroScan is a new technique to measure liver stiffness, and it has the advantages of noninvasiveness, good reproducibility and higher objectivity[6-8]. However, the FibroScan device and its maintenance are expensive, limiting the use of transient elastography in low- and middle-income countries and making it unsuitable for routine monitoring of liver fibrosis/cirrhosis in patients with chronic liver disease in economically poor areas. In recent years, researchers have been interested in finding a potential marker of liver fibrosis/cirrhosis or developing a multifactorial model from peripheral blood[9]. Noninvasive fibrosis tests based on routine laboratory indices have become available and are increasingly used to both assess and stage liver fibrosis; these include the aspartate transaminase (AST)-to-platelet (PLT) ratio index (APRI)[10], FIB-4[11] and the gamma-glutamyl transpeptidase (γ-GT) to PLT ratio (GPR)[12]. These indices are inexpensive, available and noninvasive for assessment of the stages of fibrosis/cirrhosis and can be easily performed in outpatient settings with limited conditions. Among these, the APRI was recommended by the World Health Organization as the most useful noninvasive tool to assess cirrhosis in resource-limited settings; however, FIB-4 was not recommended because it is not used for the detection of cirrhosis[13]. However, the levels of sensitivity and positive predictive value were low for APRI[14]. Therefore, there is an urgent need to find a more reliable method that is noninvasive, inexpensive, convenient and feasible for assessing liver disease stage and identifying patients who need treatment.

In this study, we tested a novel noninvasive index, the alkaline phosphatase (ALP) and γ-GT to PLT ratio (AGPR), for the assessment of liver fibrosis and cirrhosis through statistical analysis of clinical data. Moreover, we compared the diagnostic values of the AGPR, GPR, APRI and FIB-4 indices.

MATERIALS AND METHODS
Patients

The patients who participated in this study were divided into a training set and a validation set. Patients in the training set received treatment at the Affiliated Hospital of Guilin Medical University (Guilin, People's Republic of China). Patients in the validation set received treatments at the Peking University People's Hospital (Beijing, Guangxi Zhuang Autonomous Region, China). All patients were under treatment from May 2005 to October 2016, and all underwent systematic examinations, including abdominal ultrasound, routine laboratory tests and liver histological examination tests. The clinicopathologic characteristics of all patients, including age, gender, alcohol consumption, smoking status, hepatitis B e-antigen (HbeAg) status, fibrosis and cirrhosis stage, activity grade, white blood cell (WBC) count, neutrophil count (NEUT), lymphocyte count (LYMPH), PLT count, albumin, globulin, total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), AST, ALP, γ-GT and AGPR were collected and are detailed in Table 1. All patients in the study were chronically infected with HBV and were positive for hepatitis B surface antigen.

Liver histological examination
Some basic clinical examinations that ensure that patients are relatively safe when performing liver puncture surgery should be performed. In addition, physicians should obtain informed consent from the patients prior to surgery. Ultrasound localization was performed on consenting and suitable patients for liver biopsy procedures. Qualified liver tissue samples were formalin-fixed and paraffin-embedded for pathological analysis. Liver fibrosis and cirrhosis were graded as follows according to the METAVIR system: F0, no fibrosis; F1, fibrosis in the portal vein zone but no fibrous septa; F2, a small amount of fibrous septa; F3, many fibrous septa but no cirrhosis; F4, cirrhosis[15]. All biopsy samples were assessed separately by two liver pathologists who were blinded to the clinical information. If the results of their evaluation were discordant, a third highly experienced hepatopathologist blinded to patient information reviewed the contested samples. Samples were excluded from the study population if the pathologists failed to reach consensus.

Computational formula

AGPR was calculated as [ALP (U/L) + γ-GT (U/L)]/PLT count (10^9/L). APRI was calculated as (AST/ULN)/PLT count (10^9/L) × 100[10]. FIB-4 was calculated by the formula: Age (years) × AST (U/L)/[PLT (10^9/L) × ALT (U/L)$^{1/2}$][11]. The formula for GPR was γ-GT/ULN of γ-GT/PLT count (10^9/L) × 100[12].

Statistical analysis

Student's *t* test was used for continuous variables, and Pearson's χ^2 test or Fisher's exact test was used for categorical variables to compare baseline characteristics. Data are presented as the mean ± SD or proportions. Univariable logistic regression was used for the variables of age, gender, WBC, NEUT, LYMPH, PLT, albumin, globulin, TBIL, DBIL, ALT, AST, ALP, γ-GT and AGPR. The receiver operating characteristic (ROC) curves were drawn to evaluate the accuracy rate of diagnosis for the AGPR, GPR, APRI, and FIB-4. The sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio, hazard ratio and the area under the ROC curve (AUROC) of the four non-invasive markers for fibrosis and cirrhosis staging were obtained through comparison and analysis of F0-1 *vs* F2-4, F0-2 *vs* F3-4, and F0-3 *vs* F4, respectively. Data analysis was performed using SPSS software (version 24.0). A *P* value < 0.05 was regarded as statistically significant.

RESULTS

Study populations of the training and validation cohorts

Patients involved in this study needed to meet certain criteria. Those criteria were as follows: (1) Study patients were infected with HBV and underwent systematic examinations, including abdominal ultrasound, routine laboratory tests and liver biopsies; (2) Patients with HCC or other tumors were excluded; (3) Patients with coinfection of HCV, HIV or HDV were excluded; and (4) Patients with highly

controversial results on liver fibrosis pathological grading were excluded. Figure 1 displays the selection principles.

Clinical data related to this study are summarized in Table 1. There were no statistically significant differences between the training and validation cohorts in terms of age, gender, drinking status, smoking status, HbeAg, fibrosis stage, activity grade, WBC, NEUT, LYMPH, PLT, albumin, globulin, TBIL, DBIL, ALT, AST, ALP, γ-GT or AGPR ($P > 0.05$). Nonsignificant differences between the two study groups revealed that the selection of training and validation cohorts was reasonable.

AGPR predicts significant hepatic fibrosis and cirrhosis

Univariable analyses showed that the presence of significant liver fibrosis (≥ F2) was related to gender, WBC, NEUT, PLT, albumin, globulin, TBIL, DBIL, AST, ALP, γ-GT and AGPR (Table 2). In the training cohort, correlation analysis revealed a significant positive correlation between AGPR and liver fibrosis stage ($r = 0.567$, $P < 0.001$) (Figure 2A). In the validation cohort, there was also a positive correlation between AGPR and liver fibrosis stage ($r = 0.524$, $P < 0.001$), as shown in Supplementary Table 1 and Supplementary Figure 1A. Box plots showed that liver fibrosis stage positively correlated with ALP + γ-GT ($r = 0.352$, $P < 0.001$), GPR ($r = 0.509$, $P < 0.001$), APRI ($r = 0.428$, $P < 0.001$) and FIB-4 ($r = 0.416$, $P < 0.001$) in the training cohort (Figure 2B and D-F). The severity of liver fibrosis stage correlated significantly with a gradual increase in the levels of these indicators. There was a negative correlation between PLT count and liver fibrosis stage ($r = -0.362$, $P < 0.001$) in the training cohort (Figure 2C). The severity of liver fibrosis decreased with increasing PLT count. Similar results were obtained in the validation cohort, and the specific data related to these results are shown in Supplementary Figure 1B-F and Supplementary Table 2.

Comparisons of AUROC between AGPR and other noninvasive indices

The summary AUROC, sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and hazard ratios for the detection of fibrosis

and cirrhosis for AGPR, GPR, APRI and FIB-4 are displayed in Table 3. In the training cohort, the AUROC of AGPR (0.83, 95%CI: 0.78-0.87) was higher than that of GPR (0.77, 95%CI: 0.72-0.82; P = 0.008), APRI (0.72, 95%CI: 0.67-0.77; P < 0.0001) and FIB-4 (0.74, 95%CI: 0.69-0.79; P = 0.0004) for the prediction of significant fibrosis (≥ F2). For the assessment of extensive fibrosis (≥ F3), the AUROC of AGPR (0.84, 95%CI: 0.79-0.88) was also higher than that of GPR (0.81, 95%CI: 0.76-0.85; P < 0.0001), APRI (0.70, 95%CI: 0.64-0.75; P < 0.0001) and FIB-4 (0.75, 95%CI: 0.69-0.80; P = 0.0005). For the diagnosis of cirrhosis (F4), the AUROC of AGPR was 0.87 (95%CI: 0.83-0.91), which was higher than that of GPR (0.80, 95%CI: 0.75-0.84; P = 0.0001), APRI (0.76, 95%CI: 0.70-0.8; P = 0.0002) and FIB-4 (0.80, 95%CI: 0.75-0.84; P = 0.022) (Figure 3A and Table 3). For identifying patients with significant fibrosis and cirrhosis, the summary sensitivities of AGPR were 83.1% and 88.6%, respectively, while the summary specificities of AGPR were 73.4% and 75.4%, respectively. The sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and hazard ratios of the other noninvasive indices are detailed in Table 3. Our results revealed that AGPR had the best overall performance among these noninvasive indices.

We further evaluated the diagnostic accuracy and performance of these noninvasive indices in the validation cohort. Similar to the results from the training cohort, the AUROC of AGPR was better than that of GPR (0.78, 95%CI: 0.71-0.83), APRI (0.77, 95%CI: 0.70-0.83), and FIB-4 (0.77, 95%CI: 0.71-0.83) (Figure 3B and Table 3).

DISCUSSION

Cirrhosis is one of the top 20 causes of disability-adjusted life years and life lost years, accounting for 1.6% and 2.1% of the global burden, respectively[16]. Cirrhosis is also the 11th most common cause of death worldwide[16]. Recently, Xu *et al*[17] provided updated guidelines for the management of liver cirrhosis in China. However, better screening for early fibrosis or cirrhosis remains a challenge. Finding an inexpensive, noninvasive, convenient, feasible and precise parameter to stage liver fibrosis is the expectation of all medical staff in China. Although liver biopsy is a good measure for the assessment of

liver fibrosis grade, many patients do not accept it, or they are not suitable for the test[18,19]. FibroScan is a noninvasive diagnostic technique and its diagnostic accuracy is high. However, its application is limited because of its high cost[20]. Consequently, the development of noninvasive assessment methods for liver fibrosis in patients with chronic HBV infection appears especially important in clinical practice.

In the present study, we developed a new simple, convenient and noninvasive index (AGPR) to predict significant liver fibrosis in chronic HBV-infected patients in China. The correlation coefficients between liver fibrosis stage and AGPR suggest that AGPR is a good test for the assessment of significant liver fibrosis and cirrhosis.

The AGPR index was established on the basis of ALP, γ-GT and PLT. These three indicators are clinical evaluations of features of fibrosis/cirrhosis and evidence of decompensation. Hepatitis is related to ALP[21,22] and γ-GT[23,24]. ALP is useful in the diagnosis of chronic liver diseases[25]. A study showed that serum ALP level was significantly different in patients with or without liver cirrhosis[26]. In a report, γ-GT was demonstrated to be an independent predictor of hepatic fibrosis[27]. The circulating PLT count has been recommended as a biomarker of hepatic fibrosis and cirrhosis[28]. Based on these findings, each of the three variables is related to the degree of liver fibrosis. The antiviral treatment, anti-inflammatory and hepatoprotective treatment have a greater impact on serum levels of aminotransferases, such as AST and ALT. However, according to clinical observation, ALP and γ-GT are regarded as less specific for liver injury than AST and ALT. The effects of antiviral therapy, anti-inflammatory therapy and hepatoprotective therapy on ALP and γ-GT are not as obvious as on AST and ALT. As a matter of fact, elevated serum γ-GT levels are strongly associated with alcohol consumption. However, the researchers have reported that the high-risk liver disease mortality due to elevated γ-GT was not affected by alcohol consumption[29].

A routine assessment of liver fibrosis stage for patients with chronic HBV infection is needed to guide management and to indicate the need for treatment. However, the diagnosis of liver fibrosis and compensated cirrhosis cannot be based on clinically obvious features. Noninvasive fibrosis tests are now increasingly used for evaluating

liver fibrosis, reducing the need for liver biopsy. AGPR may be a new promising noninvasive fibrosis test to assist in the selection of optimal candidates for antiviral therapy. The AGPR test is inexpensive, routinely available at health-care facilities, and can be performed by untrained staff. It is suitable for conventional monitoring of hepatic fibrosis and cirrhosis. The Guidelines Development Group prioritized urgent initiation of antiviral therapy for patients with cirrhosis based on APRI score > 2 in adults, regardless of ALT or HBV DNA levels[13]. However, when applying an APRI score > 2 in this study, the sensitivity for the diagnosis of cirrhosis was only 43.2% and 50.1% in the training and validation cohorts, respectively. This suggested that more than 50% of patients with cirrhosis would be incorrectly classified as not having cirrhosis, which may lead to delayed initiation of treatment. In contrast, the sensitivity of AGPR for the diagnosis of cirrhosis was high at 88.6% and 92.3% in the training and validation cohorts, respectively. Therefore, our data suggested that AGPR may be a preferred noninvasive test to detect the presence of significant fibrosis and cirrhosis. It may serve as a simple index to make treatment decisions in patients without evidence of cirrhosis in China and other resource-limited settings where HBV infection is endemic.

Our study has some limitations. First, the selection of samples was limited to a population with chronic HBV infection in China. Whether AGPR can be generalized to different geographical areas (the infection of hepatitis C virus is endemic) remains to be determined. Second, the time span of over 10 years for our selected samples was too long (from May 2005 to October 2016). Over this time period, substantial changes have taken place in terms of medical equipment and physical examination technologies, which may reduce the accuracy of our study results. Finally, there are many factors involved in liver fibrosis. The impact of various interference factors on the diagnostic accuracy of the AGPR index has not been fully evaluated. Some interference may affect the precision of our index.

CONCLUSION

In summary, the AGPR index may be an accurate noninvasive test for predicting significant liver fibrosis and cirrhosis in patients with chronic HBV infection in China. In addition, it is suitable for conventional monitoring. Therefore, for the prediction of liver fibrosis and cirrhosis, the AGPR index is a promising noninvasive marker that is worthy of further attention and research.

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