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A novel noninvasive index for the prediction of moderate to severe fibrosis in chronic hepatitis B patients

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ABSTRACT

Backgrounds: The evaluation of liver fibrosis stages is essential for the clinical management of chronic hepatitis B (CHB).
Aims: To develop and validate a novel noninvasive index for moderate to severe fibrosis (≥S2) in CHB patients.
Methods: A total of 401 CHB patients who underwent liver biopsy were divided into the training (n = 300) and validation (n = 101) cohort. Histological severity was scored using a modified Scheuer system. Clinical and laboratory assessments were collected.
Results: In the training cohort, PACG, a novel index combining the quantitative hepatitis B core antibody (qAnti-HBc), platelet count (PLT), and albumin globulin ratio (A/G), presented better diagnostic performance (AUROC = 0.814) than that of APRI (0.735, $p = 0.007$) and FIB-4 (0.749, $p = 0.014$). In the validation cohort, the AUROC of the PACG, APRI, FIB-4 and Fibroscan were 0.834, 0.806, 0.791 and 0.810, respectively. More importantly, a higher and lower cutoff of PACG for predicting ≥S2 fibrosis or not had a >90% sensitivity and specificity, with a diagnostic accuracy of 85.9%.
Conclusion: PACG is a promising noninvasive alternative to liver biopsy in CHB patients for the evaluation of moderate to severe fibrosis.

1. Introduction

Hepatitis B virus (HBV) infection is a major global health problem and it is estimated that 240 million people are chronically infected. HBV may cause progressive liver fibrosis, leading to cirrhosis and hepatocellular carcinoma [1]. Thus, assessing the degree of liver fibrosis and its progression play a central role in the clinical management of chronic hepatitis B (CHB) patients. Patients with no or mild fibrosis appear to progress slowly, while patients with at least moderate fibrosis may progress to cirrhosis and they are candidates for antiviral treatment [2]. Currently, guidelines recommend indications for treatment based mainly on serum HBV DNA, alanine aminotransferase (ALT) levels, and the severity of liver disease. The gold standard in the assessment of liver fibrosis remains to be liver biopsy (LB) [2], especially for CHB patients in the grey zone, for example, patients with ALT levels within the upper limit of normal (ULN). However, LB is an invasive procedure with a few limitations, including possible sampling error and associated morbidity [3]. Accordingly, noninvasive tests have been developed to evaluate the degree of liver fibrosis. Fibroscan is an expensive device that can easily be used with immediate results and good reproducibility, although the results may be confounded by severe inflammation and obesity [3]. Fibroscan also requires technical or expert knowledge, restricting its utility. Furthermore, FIB-4 and APRI have also been recommended to estimate hepatic fibrosis in CHB patients by the 2015 WHO guidelines [4], but their performance in assessing significant fibrosis remains controversial due to different populations and grading systems of fibrosis [5–7]. Recently, more models

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based on CHB patients have been developed [8–10]. However, none has been widely applied in clinical practice or recommended by recent guidelines. Moreover, most noninvasive models have good diagnostic accuracy only for excluding advanced fibrosis or cirrhosis [4,6], not for detecting the early stages of fibrosis. Some of these studies also lack the performance in patients with normal ALT levels [9,10]. Thus, the development of noninvasive biomarkers that can accurately indicate moderate to severe liver fibrosis is still urgently needed in the management of CHB.

Recently, Li et al. [11] demonstrated that treatment-naïve CHB patients with moderate to severe fibrosis had a significantly higher level of quantitative antibodies specific to the HBV core protein (qAnti-HBc) compared with patients with no or mild fibrosis. This indicates that the qAnti-HBc may play an important role in predicting liver fibrosis. In the present study, we investigated the relationship between liver fibrosis and several parameters, including qAnti-HBc levels, in a large cohort of well-characterized, treatment-naïve CHB patients. We developed a novel noninvasive index to discriminate CHB patients with moderate to severe liver fibrosis, even in patients with normal ALT levels.

2. Materials and methods

The study was conducted in accordance with the guidelines of the 1975 Declaration of Helsinki and it was approved by the Institutional Ethics Committee for Human Studies at Huashan Hospital, Fudan University, Shanghai, China. The study is reported according to the Liver-FibroSTARD checklist [12] (Supplementary material). All patients enrolled were provided written informed consent before their data and serum were used for the present study.

2.1. Patients

This retrospective study was carried out at Huashan Hospital of Fudan University (Shanghai, China) from January 2006 to November 2016. Consecutive CHB patients who underwent LB were included for further evaluation. CHB was defined as the persistent presence of serum hepatitis B surface antigen for more than 6 months. Patients with the following conditions were excluded: presence of other causes of liver disease, hepatocellular carcinoma, prior antiviral therapy, insufficient liver tissue for staging of fibrosis, insufficient serum sample for quantitating qAnti-HBc levels, and incomplete data on complete blood counts and liver panel.

2.2. Histological assessment of the liver

Percutaneous LB was performed using ultrasound localization. Liver samples were obtained using 16G Menghini needles. The specimens were fixed, paraffin-embedded, and fixed in formalin. Hematoxylin-eosin and reticular fiber staining or Masson's staining were performed on each section. Patients with liver specimens shorter than 15 mm were excluded from our analysis. The mean number of portal tracts among our biopsies was 9 (range: 6–18) and the mean length was 28 mm (range: 17–36 mm). The staging of fibrosis (S0-4) was analyzed according to a modified Scheuer scoring system by two experienced clinical pathologists who were unaware of patient characteristics [13]. Moderate to severe fibrosis was defined as $S \geq 2$ and cirrhosis was defined as S4. $S < 2$ was considered to indicate no or mild fibrosis.

2.3. Fibroscan

Liver stiffness measurement (LSM) was performed in fasting conditions using FibroScan® 502 (Echosens, Paris, France), equipped with a standard M probe, by an experienced clinician according to the manufacturer's protocol [14]. The operator was blinded to the clinical characteristics of the patients. LSMs were expressed in kilopascals (kPa), and the median value was used to represent liver stiffness. The LSM value was considered to be reliable if 10 valid acquisitions were obtained and the interquartile range/median (IQR/M) was ≤30% or IQR//M >30% when the median LSM was <7.1 kPa [15]. LSM failures were not included in the analysis. LSMs of all of the patients were operated within two weeks of LB.

2.4. Clinical and laboratory assessment

Serum samples used for qAnti-HBc evaluation were taken on admission and stored at −80 °C. The qAnti-HBc levels were detected using a newly developed double-sandwich immunoassay (Wantai, Beijing, China), validated by WHO standards [16]. Other clinical and laboratory parameters were recorded and measured on admission, including age, gender, HBeAg status, HBV DNA, hepatitis B surface antigen (HBsAg), ALT, aspartate aminotransferase (AST), platelet count (PLT), prothrombin time (PT), total bilirubin (TB), albumin globulin ratio (A/G), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT). The complete blood counts were detected using Sysmex XN-9000™ (Sysmex Corporation, Kobe, Japan). Serum HBsAg, HBV DNA levels and hepatitis serology were determined as previously described [17]. The ULN of ALT was based on the Abbott ARCHITECT i2000 platform (Abbott, Chicago, IL, USA) at Huashan Hospital, 50 U/L in men and 40 U/L in women. Normal ALT was defined as an ALT level ≤ ULN.

2.5. Calculation of FIB-4 and APRI index

FIB-4 and APRI were calculated based on clinical and laboratory parameters measured at enrollment, usually one week prior to the LB.

The calculation formulae were as follows:

$$\text{FIB-4} = \text{age [years]} \times \text{AST [IU/L]} / (\text{platelet count } [\times 10^9/\text{L}] \times \text{ALT}^{1/2} \text{ [IU/L]}).$$

$$\text{APRI} = (\text{AST [IU/L]}/\text{ULN})/\text{platelet count } [\times 10^9/\text{L}] \times 100.$$

2.6. Statistical analysis

Data analysis was performed using SPSS V.19.0 (SPSS Inc., IL, USA) and MedClacV.11.4 software (MedCalc, Mariakerke, Belgium). Quantitative data were expressed as mean ± SD or median (P25, P75), assessed by the unpaired *t* test or Mann–Whitney *U* test as appropriate. The categorical data were expressed as proportions and analyzed using $\chi 2$ tests. Correlations were evaluated by the Spearman correlation coefficient (r). Univariable and multivariable analyses were used to select independent predictors of significant fibrosis. Multivariable logistic regression analysis was used to develop an index for predicting significant fibrosis. A formula was derived using the independent predictors of the final model. The receiver operating characteristic (ROC) curve was used to assess the diagnostic performance of the established fibrosis index and our new index. The area under the ROC (AUROC) curve provides an estimation of the accuracy of the tests, with higher values indicating a high level of accuracy. The AUROCs were compared using the method of DeLong et al. [18]. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) of different cutoff values were calculated to evaluate the performance of the fibrosis predictive indexes. All statistical tests were two-tailed,

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