Evaluation of non-invasive methods in hepatitis B virus (HBV)-infected patients with normal liver function

Xu Li¹, Shuang Zheng², Hongqin Xu¹³, Pujun Gao¹

Departments of ¹Hepatology and ²Radiology, The First Hospital of Jilin University, Changchun, Jilin, China; ³Jilin Province Key Laboratory of Infectious Disease, Laboratory of Molecular Virology, Changchun, Jilin, China

Abstract: Background: We aimed to compare the diagnostic performance of four non-invasive methods in detecting liver injury in chronic hepatitis B (CHB) patients with persistently normal liver function, and to develop a combined algorithm to improve current assays for liver injury evaluation. Materials and Methods: We obtained the results of aspirate aminotransferase (AST)-to-platelet ratio index (APRI), fibrosis index based on the 4 factors (FIB-4), red blood cell distribution width (RDW) to platelets ratio (RPR) and Fibroscan in a cohort of 58 CHB patients who underwent liver biopsy (LB). Then we combined serum markers with Fibroscan and evaluated their performance in detecting significant fibrosis. Results: The areas under the receiver operating characteristic curve (AUROC) for APRI, FIB-4, RPR and Fibroscan were 0.696, 0.708, 0.736 and 0.756 respectively for detecting significant liver fibrosis. An improved performance was obtained by combining Fibroscan and RPR, AUROC of whom was 0.836, reducing liver biopsy required for detection of significant fibrosis in 37.9% of patients with an accuracy of 95%. Conclusion: APRI, FIB-4, RPR and Fibroscan show moderate clinical value for detecting significant fibrosis in chronic HBV patients with normal liver function. The combination of Fibroscan and RPR improved diagnostic performance and reduced the number of patients who need liver biopsy.

Keywords: Aspirate aminotransferase-to-platelet ratio index, fibrosis index based on the 4 factors, red blood cell distribution width to platelets ratio, Fibroscan, Hepatitis B virus, normal liver function

Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. Approximately 240 million people are chronically infected with HBV, and are at high risk for developing cirrhosis and hepatocellular carcinoma (HCC) [1]. And chronic HBV infection causes a wide spectrum of clinical manifestations. The guidelines for managing HBV infection do not recommend antiviral treatment for patients with normal alanine aminotransferase (ALT) levels. There is an increasing concern about accuracy to reflect liver injury by ALT. Recent studies have found significant necroinflammation and/or fibrosis in 28-37% of HBV patients with persistent normal ALT [2, 3]. High risk for advanced fibrosis and cirrhosis in patients can be delayed or prevented by antiviral therapy if significant fibrosis is detected earlier [4, 5].

Liver biopsy (LB) based evaluation of liver histology has been the most reliable detection of liver inflammation, stage of liver fibrosis and treatment efficiency. However LB is invasive, expensive and inconvenient for patients. Furthermore, sampling error occurs if histological alterations are non-homogenous, and variations between intra and inter-observers pose another challenge [6-9].

There is a strong interest in the field for developing non-invasive methods for accurately detecting liver injury. Fibroscan, a new imaging technique, measures the velocity of an elastic shear wave through the liver that reflects liver stiffness, resulting in an estimate of liver fibrosis [10, 11]. Some researchers have developed a mathematical formula that calculates a value for fibrosis using routine laboratory tests in chronic hepatitis C (CHC). Aspirate aminotransferase (AST)-to-platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4) are examples of routine serum tests [12-14]. Previous analyses suggested that FIB-4 and APRI are strong predictors for liver fibrosis in
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HBV-infected patients with elevated ALT [15, 16]. Red blood cell distribution width (RDW) to platelets ratio (RPR) is an index of platelet morphology, which can predict significant fibrosis and cirrhosis in CHB patients with relatively high accuracy [17, 18].

However, few studies evaluated detection of significant fibrosis in HBV patients with PNALT using Fibroscan, APRI, FIB-4 and RPR. The aim of this study was to evaluate these four non-invasive assays for detecting significant liver fibrosis using liver histology as a reference in CHB patients with persistent normal liver function. We also tested the models that combined different non-invasive methods to evaluate whether the combination can decrease the rate of LB and have a high accuracy for detecting significant fibrosis in these patients.

Materials and methods

Patients

We retrospectively enrolled 58 patients who were previously diagnosed as CHB and underwent LB from January 2010 to December 2015 in the First Hospital of Jilin University. The purpose of LB was to assess severity of liver fibrosis and inflammation and to determine whether antiviral treatment is warranted. Inclusion criteria were as follows: 1) Patients were hepatitis B surface antigen (HBsAg) positive for at least 6 months and chronic hepatitis was confirmed by histology, HBV DNA positive (HBV DNA > 50 IU/ml by PCR); 2) Liver function was normal, as determined by ALT, AST, and total bilirubin (TBIL), for at least two consecutive measurements over a period of 6 months [19], the upper limit of normal (ULN) of ALT, AST and TBIL are 50 U/L for male/40 U/L for female, 40 U/L for male/35 U/L for female and 30 µmol/L, respectively; 3) Age of patients ≥ 18 years old. Exclusion criteria were: 1) Patients who were co-infected with hepatitis C virus or human immunodeficiency virus (HIV), auto-immune hepatitis, Wilson disease, hepatocellular carcinoma, chronic ethanol consumption (> 20 g/day for female, > 40 g/day for male). 2) A history of previous antiviral treatment or a history of malignant disorder. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee in the First Hospital of Jilin University.

Liver biopsy examination

Percutaneous LB was using an 18 G biopsy needle. LB was repeated for patients whose liver tissues were shorter than 13 mm to minimize the influence of the length of liver specimen on the accuracy of diagnosis. The liver tissues were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. Every specimen was assessed by one pathologist who was blinded to clinical data. The Scheuer scoring system was used to evaluate the samples. Fibrosis was therefore scored on a scale from 0 to 4 (S0 = no fibrosis, S1 = enlarged portal tracts, S2 = periportal or portal septa, S3 = fibrosis with architectural distortion, S4 = cirrhosis). Significant fibrosis was defined as Scheuer score ≥ 2 [20].

Liver stiffness measurement by Fibroscan

Fibroscan (Fibroscan, Echosens SA, Paris, France) was performed by an experienced operator (more than 100 determinations in patients with chronic liver diseases) within 1 week before LB. Per instruction, 10 validated measurements were taken for each patient. The median value (in kilopascal, kPa) was considered representative of the liver elastic modulus. The measurement was considered reliable unless the interquartile range/median was < 30% and success rate was > 60% as suggested by the manufacturing company [21].

Non-invasive biomarkers

Fasting blood serum samples were used for laboratory tests within one week prior to LB. Platelet (PLT), RDW, AST, ALT, TBIL and gamma-glutamyl transpeptidase (GGT) were analyzed. HBV DNA level was determined with real time PCR with low detection limit of 50 IU/ml. HBsAg, anti-HBsAb, hepatitis B e-antigen (HBeAg), anti-HBe, hepatitis core B antibody (HBcAb), anti-HCV and anti-HIV were also assessed. APRI, FIB-4 and RPR were calculated using the principle reported formulas, as APRI = AST (U/L)/[PLT (10^9/L)], FIB-4 = Age (years) × AST (U/L)/[PLT (10^9/L) × ALT^{1/2} (U/L)], RPR = RDW/PLT (10^9/L).

Cut-off values

Cut-off values of APRI, FIB-4, and RPR were selected from the published literatures while
the cut-off value of Fibroscan was derived from the Manual [12-14]. Patients with APRI > 0.5, FIB-4 > 1.45, RPR > 0.1 or Fibroscan ≥ 7.3 kPa were considered to have no significant fibrosis.

Evaluation of the combined non-invasive methods

Evaluation of non-invasive methods was designed for better detection of significant fibrosis by combining serum markers with Fibroscan, and the results were compared with histology diagnosis. In algorithm APRI+Fibroscan, if the results of APRI and Fibroscan were consist (APRI < 0.5 and Fibroscan < 7.3 kPa or APRI > 0.5 and Fibroscan ≥ 7.3 kPa), the patients were classified as significant fibrosis free(< S2) or to be positive for significant fibrosis (≥ S2). If the results were inconsistent, the patients were considered to need LB. The other algorithms were similar with APRI+Fibroscan.

Statistical analysis

All statistical analyses were processed using SPSS, version 22.0 (SPSS Inc, Chicago, IL). Continuous variables are expressed as mean ± standard deviations (SD) and the difference was determined by student t test. Categorical variables are expressed as percentages and the difference was determined by the chi-square test. A P value < 0.05 was considered significant. The diagnostic performance of APRI, FIB-4, RPR and Fibroscan and their combinations were assessed by sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic accuracy, receiver operating characteristic curves (ROC) and AUROC. McNemar’s test was performed to evaluate the agreement between the combination with best accuracy and liver histology.

Results

Characteristics of enrolled patients

A total of 58 patients were enrolled in this study. The demographic, laboratory, Fibroscan results and histological features are summarized in Table 1. Median years of HBV infection were 11.6, 44.8% of the patients were HBeAg positive. There was no significant difference in the years of HBV infection (P = 0.74) between HBeAg positive and negative patients. Significant fibrosis was detected in 37.3% patients with normal liver function, 8.6% of whom had liver cirrhosis.

Performance of individual non-invasive methods

The diagnostic performance of the four non-invasive methods were evaluated for their ability to detect significant fibrosis (Scheuer fibrosis stage ≥ S2). Cut-off values for APRI, FIB-4, RPR and Fibroscan were 0.5, 1.45, 0.1 and 7.3 kPa respectively. RPR had the highest specificity, LR+, diagnostic accuracy and the lowest LR-(Table 2). ROC curves of these methods for detecting significant fibrosis were presented in Figure 1, AUROC of APRI, FIB-4, RPR and
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Fibroscan were 0.696, 0.708, 0.736 and 0.756, respectively.

Table 2. Performance of APRI, FIB-4, RPR and Fibroscan in excluding significant fibrosis in livers

<table>
<thead>
<tr>
<th>Method</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>LR+</th>
<th>LR-</th>
<th>DA (%)</th>
<th>AUROC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRI</td>
<td>0.5</td>
<td>31.8</td>
<td>86.1</td>
<td>2.29</td>
<td>0.79</td>
<td>65.5</td>
<td>0.696 (0.55-0.84)</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.45</td>
<td>59.1</td>
<td>80.6</td>
<td>3.05</td>
<td>0.51</td>
<td>72.4</td>
<td>0.708 (0.56-0.86)</td>
</tr>
<tr>
<td>RPR</td>
<td>0.1</td>
<td>45.5</td>
<td>91.7</td>
<td>5.48</td>
<td>0.02</td>
<td>74.1</td>
<td>0.736 (0.62-0.89)</td>
</tr>
<tr>
<td>Fibroscan</td>
<td>7.3</td>
<td>44.4</td>
<td>86.4</td>
<td>3.26</td>
<td>0.64</td>
<td>60.3</td>
<td>0.756 (0.60-0.87)</td>
</tr>
</tbody>
</table>

APRI: aspirate aminotransferase to platelet ratio index; FIB-4: fibrosis index based on the 4 factors; RPR: red blood cell distribution width to platelets ratio; LR+: positive likelihood ratio; LR-: negative likelihood ratio; DA: diagnostic accuracy; AUROC: area under receiver operating characteristic curve.

Figure 1. AUROC curves for fibrosis scores (APRI, FIB-4, RPR, Fibroscan) at different stages of fibrosis: S0-1 vs S ≥ 2, with an area under the receiver operating characteristic curve (AUROC) of 0.696, 0.708, 0.736 and 0.756 respectively.

Table 3. Performance of combinations of APRI, FIB-4, RPR and Fibroscan in detecting significant fibrosis in livers

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>LR+</th>
<th>LR-</th>
<th>DA (%)</th>
<th>AUROC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRI+Fibroscan</td>
<td>70.0</td>
<td>82.4</td>
<td>3.98</td>
<td>0.25</td>
<td>77.8</td>
<td>0.779 (0.65-0.91)</td>
</tr>
<tr>
<td>FIB-4+Fibroscan</td>
<td>91.7</td>
<td>80.0</td>
<td>4.59</td>
<td>0.10</td>
<td>85.2</td>
<td>0.804 (0.68-0.93)</td>
</tr>
<tr>
<td>RPR+Fibroscan</td>
<td>100.0</td>
<td>93.3</td>
<td>14.93</td>
<td>0.00</td>
<td>95.5</td>
<td>0.836 (0.73-0.94)</td>
</tr>
</tbody>
</table>

APRI: aspirate aminotransferase to platelet ratio index; FIB-4: fibrosis index based on the 4 factors; RPR: red blood cell distribution width to platelets ratio; DA: diagnostic accuracy; AUROC: area under receiver operating characteristic curve.

Optimal combination algorithms

After individual evaluations of the four non-invasive methods, we further explored paired combination of these methods. AUROC for predicting significant fibrosis were 0.779, 0.804, 0.836, respectively (Table 3). RPR+ Fibroscan had the highest AUROC (Figure 2), meanwhile the combination improved the performance to a better level than that achieved by RPR or Fibroscan alone (ROCs: 0.836 vs 0.736 or 0.756). RPR+ Fibroscan was the best performing algorithm with sensitivity, specificity, LR+, LR-, accuracy of 100%, 93.3%, 14.94, 0, 95.5%, respectively. However the consistency of RPR and Fibroscan was mediocre, with only 37.9% of the patients. Next, we performed McNemar’s test to compare the agreement between RPR+Fibroscan and LB. There was an almost perfect agreement (Kappa = 0.899, P < 0.001) between RPR+ Fibroscan and LB.

RPR and Fibroscan agreed on the diagnosis of the stage of fibrosis in 22 patients (37.9%). There was only 1 discordant case who was classified as ≥ S2 by RPR+4+Fibroscan but < S2 by LB. Among the remaining 36 patients, Fibroscan agreed with LB results in 14 cases and RPR agreed with LB in 22 cases. The best
diagnostic algorithm for these six combinations is reported in Figure 3.

Discussion

In this study, we evaluated clinical values of four non-invasive methods, APRI, FIB-4, RPR and Fibroscan as well as paired combinations in a cohort of 58 CHB patients with persistent normal liver function. We found that a combination of RPR+Fibroscan can detect significant fibrosis with a good accuracy of 95.5%, as confirmed by liver biopsy.

High prevalence of histologically significant necroinflammation or fibrosis in HBV patients with PNALT has been reported [2, 3]. Considering ALT level cannot reflect significant fibrosis correctly, we rely on additional non-invasive markers and LB to detect fibrosis. The diagnostic performance of the four non-invasive methods tested in this study was compared based on AUROC and diagnosis accuracy. RPR showed a better diagnostic accuracy among the three serum biomarkers. The best AUROC of these serum biomarkers was 0.736, diagnostic accuracy was 74.1%, which was lower than the values reported by previous studies [15, 16, 22]. The differences can be explained by varying degrees of fibrosis since AUROC is highly influenced by the prevalence of fibrosis stage. The percentage of S0-1 detected in our study was higher compared to other studies. Furthermore, the diagnostic performance of serum biomarkers in PNALT patients was not as good as in individuals with elevated ALT levels [23-26]. To our knowledge, this study represents the first validation of RPR diagnostic value in HBV patients with normal liver function, and RPR showed the best diagnostic performance among other serum biomarkers.

We explored different non-invasive detection combinations of serum markers and Fibroscan to reduce liver biopsy in CHB patients. Among the four non-invasive methods and paired combinations, the combination of Fibroscan and RPR revealed the best diagnostic performance for significant fibrosis. This combination scan...
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covered 37.9% of the cohort patients, 95.5% of patients with S ≥ 2 were detected by Fibroscan+RPR, as well as histology. RPR consists of two common hematological parameters and is easy to calculate. Fibroscan presents no risk compared to liver biopsy. Thus, this non-invasive method has potential for wide clinical applications.

To increase the diagnostic accuracy, many combinations of non-invasive methods have been proposed. The resultant diagnostic accuracies ranged from 84 to 94%, and reduced LB by 48 to 77% in patients with significant fibrosis [27-31]. Since elevated ALT influences the accuracy of serum biomarkers and Fibroscan [25, 26, 32, 33], only patients with normal liver function were selected in this study. We expect that our results could be validated in large cohorts. Furthermore, this study is the first to report that RPR evaluated in patients with normal liver function and used in a combination with another non-invasive method, resulting in impressive accuracy.

There are a few limitations in this study. The enrolled patients in a retrospective study may have been subjected to a biased selection. And the number of patients was small because only HBV-infected patients with normal liver function were included. The main findings need to be verified in large cohorts.

In conclusion, we evaluated the diagnostic performance of four non-invasive methods in HBV patients with normal liver function, and found similar diagnostic performances among these four. A combination of RPR and Fibroscan can improve the performance of each individual non-invasive method, and help predict significant fibrosis in 40% of patients without LB.

Disclosure of conflict of interest

None.

Address correspondence to: Pujun Gao, Department of Hepatology, The First Hospital of Jilin University, Jilin University, No. 71, Xinmin Street, Changchun 130021, Jilin, China. Tel: +86 187 4301 2103; E-mail: gjp0411@163.com

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