The diagnostic value of a globulin/platelet model for evaluating liver fibrosis in chronic hepatitis B patients

Banu Demet Coskun¹, Engin Altinkaya¹, Eylem Sevinc², Mustafa Ozen³, Hatice Karaman⁴, Ahmet Karaman⁴ and Orhan Poyrazoglu¹

Departments of ¹Gastroenterology, ²Pediatric Gastroenterology, ³Internal Medicine, and ⁴Pathology. Kayseri Training and Research Hospital. Kayseri, Turkey

ABSTRACT

Background: Liver biopsy, which is considered the best method for evaluating hepatic fibrosis, has important adverse events. Therefore, non-invasive tests have been developed to determine the degree of hepatic fibrosis in patients with chronic hepatitis B.

Aim: To verify the usefulness of a new fibrosis index, the globulin/platelet model in patients with chronic hepatitis B and to compare it with other noninvasive tests for predicting significant fibrosis. This study was the second to evaluate the globulin/platelet model in HBV patients.

Methods: We retrospectively investigated 228 patients with chronic hepatitis B who performed liver biopsy from 2013 to 2014. The globulin/platelet model, APGA [AST/Platelet/Gamma-glutamyl transpeptidase/Alfa-fetoprotein], FIB4, fibrosis index, cirrhosis discriminate score, and Fibro-quotient were calculated, and the diagnostic accuracies of all of the fibrosis indices were compared between the F0-2 (no-mild fibrosis) and F3-6 (significant fibrosis) groups.

Results: All of the noninvasive markers were significantly correlated with the stage of liver fibrosis (p < 0.001). To predict significant fibrosis (F ≥ 3), the area under the curve (95% CI) was found to be greatest for APGA (0.83 [0.74-0.86]), followed by FIB-4 (0.75[0.69-0.80]), the globulin/platelet model (0.74 [0.68-0.79]), fibrosis index (0.72 [0.6-0.78]), cirrhosis discriminate score (0.71 [0.64-0.76]) and Fibro-quotient (0.62 [0.55-0.7]). The area under the receiver operating characteristic curves of APGA was significantly higher than that of the other noninvasive fibrosis markers (p < 0.05).

Conclusions: While the APGA index was found to be the most valuable test for the prediction significant fibrosis in patients with chronic hepatitis B, GP model was the thirtieth valuable test. Therefore, we recommended that APGA could be used instead of the GP model for prediction liver fibrosis.

Key words: Chronic hepatitis B. Liver fibrosis. Noninvasive fibrosis marker. Globulin/platelet model.

INTRODUCTION

Hepatitis B virus (HBV) infection is the most common cause of chronic liver disease, which can result in many serious complications, such as cirrhosis, hepatocellular carcinoma (HCC) and death. In untreated patients with chronic hepatitis B (CHB), the cumulative incidences of cirrhosis, hepatic decompensation and hepatocellular carcinoma at 5 years were approximately 8-20%, 15% and 2-5% (1,2), respectively. The current guidelines for HBV management propose liver biopsy to begin antiviral therapy and to obtain prognostic information. The presence of significant fibrosis indicates the need for antiviral therapies. Once diagnosed, fibrosis should be treated as early as possible using appropriate methods (3-5).

Histopathologic examination of the liver is the gold standard for the assessment of liver fibrosis. However, liver biopsy is an invasive procedure and is not readily repeatable for the assessment of post-treatment liver fibrosis. It also results in sampling errors and intra-interpretation variability (6,7). For these reasons, numerous noninvasive markers have been derived from independent indicators of liver fibrosis. These markers include AAR (aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio), APRI (AST/platelet ratio index), API (age-platelet index), APGA [AST/Platelet/Gamma-glutamyl transpeptidase (GGT)/Alfa-fetoprotein (AFP)] (8), CDS (cirrhosis discriminate score) (9), FI (Fibrosis index) (10), Göteborg University cirrhosis index (GUCI) (11) and Fibro-quotient (Fibro Q) (12). The GP model (including globulin and platelets) is a newly developed model for predicting significant liver fibrosis in patients with CHB (13). To date, only one study has evaluated the GP model in association with significant fibrosis in patients with CHB. However, this newly designed noninvasive marker must be evaluated in different populations before common use.

Therefore, we compared and evaluated the diagnostic accuracies of six markers of hepatic fibrosis, including the GP model, APGA, FI, FIB-4, Fibrosis Q and CDS, in the prediction of significant fibrosis in patients with CHB from the Turkish population.
MATERIAL AND METHODS

Patients

We retrospectively examined the computerized files and liver biopsies of 250 treatment-naive patients with CHB who performed percutaneous liver biopsy at the Kayseri Training and Research Hospital, Department of Gastroenterology from January 2012 to December 2014. All of the patients were positive for HBsAg for more than 6 months and had HBV-DNA levels greater than 10^4 IU/L. The exclusion criteria were co-infection with hepatitis C or HIV, hepatitis delta superinfection, use of antiviral medicine, decompensated cirrhosis, liver cancer, a history of alcohol use (> 20 g/day), concomitant chronic liver disease, such as nonalcoholic steatohepatitis, autoimmune hepatitis, or Wilson disease, and conditions that might affect liver function tests and platelets, non-representative liver biopsy (portal fields < 6). Twenty two patients who have nonrepresentative liver biopsies were excluded from the study. The final analysis was performed in 228 patients. The study protocol was permitted by the local ethics committee, and informed consent was taken from the patients.

Methods

The clinical and laboratory parameters were recorded from each patient at the time of liver biopsy. HBsAg, hepatitis Be antigen (HBcAg), antibodies to HBcAg and HDV and biochemical tests for AST, ALT, gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin, albumin, complete blood count, prothrombin time (PT) and international normalized ratio (INR) were performed using commercial assays in the clinical laboratory. The viral load was determined using a quantitative real-time polymerase chain reaction (PCR) system and was expressed as log10 IU/mL. The following serum biomarkers of fibrosis were calculated as previously described in the literature: GP model, APGA, FIB-4, FI, CDS and fibro Q. The formulas of the indirect fibrosis markers are shown in table I. All of the biopsies were obtained with a 16 G Menghini type needle. Liver biopsy samples were reviewed by a single pathologist blinded to the patient details and clinical data. The stage of fibrosis was measured, according to the Ishak fibrosis scoring system (11). Biopsy specimens with at least six portal fields were considered representative. We further grouped the fibrosis stages as F0-2 (non-minimal fibrosis) and F3-6 (significant fibrosis).

Statistical analysis

Data normality was tested using the Shapiro-Wilk test. Levene’s test was used to assess the variance of homogeneity. For independent samples, Student’s t-test and the Mann-Whitney U test were used to compare the differences between continuous variables, and Pearson’s chi-square analysis was used to compare categorical variables. Nonparametric ROC (receiver operating characteristics) analyses were applied for non-invasive serum biomarkers and area under the curve (AUC) measurements were calculated with 95% confidence intervals and were compared to one another. Cutoff values were calculated for each biomarker using Youden’s index, and sensitivity, specificity, positive predictive rate, and negative predictive rate diagnostic measurements were calculated with 95% confidence intervals. The analyses were conducted using R software (www.r-project.org), version 3.1.0, with p < 0.05 considered to be statistically significant. MedCalc for Windows, version 9.38 (MedCalc Software, Mariakerke, Belgium), was used for pairwise comparison of ROC curves.

RESULTS

Patient characteristics

A total of 228 patients with CHB (mean age 46.2 ± 12.5 years; 67% male) were included in this study. Of the 228 patients, 113 (49.6%) had no or mild fibrosis (F0-2), and 115 (50.4%) had significant fibrosis (F3-6). The mean liver specimen length was 16 ± 0.8 mm. There was no correlation between the Ishak fibrosis stage and liver specimen length (r = -0.59, p = 0.6). We compared the diagnostic accuracies of all of the fibrosis indices between the F0-2 (no-mild fibrosis) and F3-6 (significant fibrosis) groups. The comparisons of demographic and clinical characteristics with non-invasive serum biomarkers between fibrosis stages in CHB patients are shown in table II.

Results of correlation analysis between Ishak fibrosis stage and indirect markers of fibrosis

We analyzed the correlations between the values of noninvasive models and fibrosis scores, to evaluate whether noninvasive models are suitable for staging liver fibrosis in CHB patients. Strong correlations were observed for APGA (r = 0.53), GPl (r = 0.42) and, FIB-4 (r = 0.41) (p < 0.001). Moderately correlations were observed for FI (r = 0.33) and CDS (r = 0.36) (all p < 0.001), and a weak correlation was
observed for fibro Q ($r = 0.22$) ($p < 0.001$). The results of the correlations among routine blood tests, noninvasive fibrosis markers and fibrosis stages are summarized in table III.

**ROC curve analysis**

We further investigated whether the non-invasive models could distinguish between significant fibrosis and cirrhosis. ROC curves were constructed for each model.

To predict significant fibrosis ($F \geq 3$), the AUC (95% CI) was found to be greatest for APGA ($0.83 [0.74-0.86]$), followed by FIB4 ($0.75 [0.69-0.80]$), the GP model ($0.74 [0.68-0.79]$), FI ($0.72 [0.6-0.78]$), CDS ($0.71 [0.64-0.76]$) and Fibro Q ($0.62 [0.55-0.77]$). The AUROC of APGA was significantly greater than that for the other fibrosis markers ($p < 0.001$). Comparisons of the ROC curves for the diagnostic accuracies of APGA, FIB-4, FI, CDS and Fibro Q in identifying fibrosis stages F0-2 and F3-6 in CHB are plotted in figure 1.

The sensitivity, specificity, and positive/negative predictive values were calculated based on the cutoff points determined for the seven indirect indices, a summary of which is provided in table IV.
LIVER FIBROSIS IN CHRONIC HEPATITIS B PATIENTS

Our results revealed that the second most valuable test after the PAPAS index for detecting significant fibrosis was the APGA index, having a sensitivity of 16.9%, a specificity of 91%, a PPV of 68.2% and an NPV of 81.3% at a cutoff value of ≤ 6.16. At a cutoff value of ≤ 6.16, the APGA index had sensitivity of 81%, specificity of 68.5%, a PPV of 68.2% and an NPV of 81.3% for significant fibrosis. These results were similar to those of previous published studies.

FIB-4 has been mainly evaluated in HCV-mono-infected or HIV-HCV-co-infected patients, generating favorable outcomes. These studies demonstrated AUROC for the FIB4 index of 0.85 and 0.91, respectively (15,16). Kim et al. (17) first evaluated the diagnostic value of the FIB-4 index for predicting fibrosis in 689 CHB patients and compared it with APRI, AAR, API and SPRI (spleen-to-platelet ratio index). They detected that the AUROCs of FIB-4 for the prediction of significant fibrosis (F ≥ 2), severe fibrosis (F ≥ 3) and cirrhosis (F4) were 0.865, 0.910 and 0.926, respectively. Ma et al. (18) in study of 1168 cases of CHB, evaluated whether seven noninvasive models, including FIB-4, AAR, API, Fibro Q, CDS and Lock’s model, were suitable for staging liver fibrosis, and they found that FIB-4 and Lock’s model were the most effective models for distinguishing significant and extensive fibrosis. When the cutoff value was 1.433-1.858, FIB-4 showed sensitivity of 94%, specificity of 45.8%, a PPV of 67.3%, and an NPV of 86.6%. In our study, the FIB4 index was the second most valuable test for the prediction of significant fibrosis, with an AUROC value of 0.75, an NPV of 72%, a PPV of 64% and sensitivity of 68%. We observed lower statistical values than those from the above studies for excluding significant fibrosis, which might have been related to the use of different staging systems (the META VIR vs. the Ishak system), the differences in patient populations or the prevalence of significant fibrosis.

The GP model is a new designed non-invasive fibrosis marker. To date, there has been only one study that has evaluated the GP model to predict significant fibrosis in CHB patients. Liu et al. (13) derived and validated the GP model in a cohort of 114 patients with CHB. They reported that the AUROC of the GP model for the predicting significant fibro-

### Table IV. Diagnostic measures and AUROC results of noninvasive fibrosis markers in the detection of significant fibrosis in CHB patients

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cutoff</th>
<th>AUROC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI</td>
<td>&gt; 1.5</td>
<td>0.74</td>
<td>75.2 (65.2-83.6)</td>
<td>62.8 (53.2-71.7)</td>
<td>62</td>
<td>75</td>
</tr>
<tr>
<td>FIB4</td>
<td>&lt; 1.45</td>
<td>0.75</td>
<td>68 (57.7-77.3)</td>
<td>68.1 (58.7-76.6)</td>
<td>66</td>
<td>68</td>
</tr>
<tr>
<td>GPI</td>
<td>&gt; 1.5</td>
<td>0.74</td>
<td>75.2 (65.2-83.6)</td>
<td>62.8 (53.2-71.7)</td>
<td>62</td>
<td>75</td>
</tr>
<tr>
<td>FI</td>
<td>&lt; 2</td>
<td>0.72</td>
<td>52.7 (42-63)</td>
<td>83.2 (75.9-6)</td>
<td>72.1</td>
<td>68.1</td>
</tr>
<tr>
<td>CDS</td>
<td>4</td>
<td>0.71</td>
<td>81 (71.5-88.6)</td>
<td>68.5 (59-77)</td>
<td>68.2</td>
<td>81.3</td>
</tr>
<tr>
<td>FibroQ</td>
<td>3.73</td>
<td>0.64</td>
<td>34 (24.6-44.5)</td>
<td>90.3 (83.2-95)</td>
<td>74.4</td>
<td>62.2</td>
</tr>
</tbody>
</table>

AUROC: Area under ROC curve; NPV: Negative predictive value; PPV: Positive predictive value; CDS: Cirrhosis discriminate score; FI: Fibrosis index; FibroQ: Fibro-quotient; FIB-4: Fibrosis index based on the four factors; GP model: Globulin/platelet model.

**DISCUSSION**

Our study aimed to identify patients with and without significant fibrosis using non-invasive fibrosis markers, such as the GP model, APGA, FIB-4, FI, CDS, and Fibro Q in patients with HBV.

We showed that all of the noninvasive markers could distinguish significant fibrosis from no-mild fibrosis with different accuracies and significances. However, APGA, FIB-4 and the GP model were found to be the most precise models for distinguishing significant fibrosis from no-mild fibrosis. The AUC (95% CI) was found to be greatest for APGA, followed by FIB4 and the GP model (0.83, 0.75 and 0.74, respectively). In addition, when we analyzed the correlations between the values of the non-invasive models and the fibrosis scores, particularly those of APGA (r = 0.53), the GP model (r = 0.42) and FIB-4 (r = 0.41) with fibrosis stage, we observed strong correlations (p < 0.001).

For FI (r = 0.369) and CDS (r = 0.352), we observed moderate correlations (p < 0.001) (all, p < 0.001).

The APGA index is a non-invasive test for the assessment of liver fibrosis, based on biochemical parameters (AST, GGT, AFP and platelet count). According to Fung et al. (8), it was first designed for predicting significant fibrosis and cirrhosis in 265 CHB patients. They found that the AUC of APGA for predicting significant fibrosis and cirrhosis was 0.85 in both the training and validation groups. Using an optimal cutoff value of 6.9 for significant fibrosis, the sensitivity was 82%, with specificity of 69% and an NPV of 91%. The AUC of the APGA index was greater than those of the AAR, APRI and API (0.85, 0.80, 0.38 and 0.68, respectively). In another study, Seto et al. (14) using a cut off < 6.687, demonstrated that the APGA index had sensitivity of 16.9%, a specificity of 91%, a PPV of 81.3%, and an NPV of 71% to exclude significant fibrosis in CHB. In this study, the APGA index was also compared to PAPAS (Platelet/Age/Phosphatase/AFP/AST) index, APRI and FIB-4. The APGA index was found to be the second most valuable test after the PAPAS index for the detection of significant fibrosis. Our results revealed that the AUROCs of the APGA index were the best among the six models. The AUROC for the APGA index for predicting significant fibrosis was 0.81 (0.74-0.86). At a cutoff value of ≤ 6.16, the APGA index had sensitivity of 81%, specificity of 68.5%, a PPV of 68.2% and an NPV of 81.3% for significant fibrosis. These results were similar to those of previously published studies.

For FI (r = 0.369) and CDS (r = 0.352), we observed correlations (p < 0.001) (all, p < 0.001).

The APGA index is a non-invasive test for the assessment of liver fibrosis, based on biochemical parameters (AST, GGT, AFP and platelet count). According to Fung et al. (8), it was first designed for predicting significant fibrosis and cirrhosis in 265 CHB patients. They found that the AUC of APGA for predicting significant fibrosis and cirrhosis was 0.85 in both the training and validation groups. Using an optimal cutoff value of 6.9 for significant fibrosis, the sensitivity was 82%, with specificity of 69% and an NPV of 91%. The AUC of the APGA index was greater than those of the AAR, APRI and API (0.85, 0.80, 0.38 and 0.68, respectively). In another study, Seto et al. (14) using a cut off < 6.687, demonstrated that the APGA index had sensitivity of 16.9%, a specificity of 91%, a PPV of 81.3%, and an NPV of 71% to exclude significant fibrosis in CHB. In this study, the APGA index was also compared to PAPAS (Platelet/Age/Phosphatase/AFP/AST) index, APRI and FIB-4. The APGA index was found to be the second most valuable test after the PAPAS index for the detection of significant fibrosis. Our results revealed

that the AUROCs of the APGA index were the best among the six models. The AUROC for the APGA index for predicting significant fibrosis was 0.81 (0.74-0.86). At a cutoff value of ≤ 6.16, the APGA index had sensitivity of 81%, specificity of 68.5%, a PPV of 68.2% and an NPV of 81.3% for significant fibrosis. These results were similar to those of previously published studies.

**AUROC:** Area under ROC curve; **NPV:** Negative predictive value; **PPV:** Positive predictive value; **CDS:** Cirrhosis discriminate score; **FI:** Fibrosis index; **FibroQ:** Fibro-quotient; **FIB-4:** Fibrosis index based on the four factors; **GP model:** Globulin/platelet model.
sis (F \geq 2) and cirrhosis were 0.732 and 0.738, respectively. Using a cutoff value of < 1.68, the GP model had sensitivity of 72.4%, specificity of 69.6%, a PPV of 71.2%, and an NPV of 70.8% for the prediction of significant fibrosis. In this study, the GP model was also compared with FL, FIB-4, fibrosis- cirrhosis index (FCI), APRI, API and AST/platelets, and the GP model showed the strongest correlation with severity of fibrosis (r = 0.441, p < 0.001). The AUROC of the GP model was not superior to other noninvasive markers (p > 0.05). In the present study, we found that the AUROC for the GP model for predicting significant fibrosis was 0.74 (0.68-0.79). At a cut-off value of \leq 1.5 for significant fibrosis, the GP model had a sensitivity of 75.2%, a specificity of 62.8%, a PPV of 62% and an NPV of 75% for significant fibrosis. In addition, when the GP model was compared with other five noninvasive markers, the GP model showed the third highest correlation with significant fibrosis after APGA index and FIB-4. However, there were no significantly different between the AUROC of FIB4 and GP model (p > 0.05). Using values below the lower cutoff level (1.5), a presence of significant fibrosis could be predicted in 62.8% of patients. In our study, these variable results may be related to use of different histopathologic scoring system (the METAVIR vs. the Ishak system) and number of patients.

In earlier studies, serum globulin, immunoglobulin levels and platelets demonstrated high predictive value for the extent of hepatic fibrosis in patients with CHB and CHC (13,19). Schmilovitz-Weiss et al. (20) reported that serum globulin levels were the strongest predictors of severe fibrosis (OR 5.97, 95% CI 1.82-19.53, p = 0.0004), followed by platelet count (OR 0.98, 95% CI 0.97-0.99, p: 0.001) and serum Ig G level (OR 1.003, 95% CI 1.000-1.007, p < 0.042). In addition, they reported that there was a 0.5 point increase in the stage of hepatic fibrosis, for each increase of 0.33 mg/dL in serum globulin. In this study, while hepatic fibrosis positively moderate correlated with serum globulin level, inversely moderate correlation was found with platelets (r = 0.34 and r = -0.34, p < 0.001, respectively). In addition, while the median globulin level in CHB patients with significant fibrosis was significantly higher than in CHB patients with no-minimal fibrosis, the platelet count was significantly lower than no-minimal fibrosis (all, p = 0.001). Our results supported that serum globulin level increased, while platelets gradually decreased with fibrosis progression (19,20).

In conclusion, our study showed that the GP model showed limited value in identify hepatitis B related significant fibrosis. The APGA index was found to be the most valuable test with a %81.3 NPV, FIB-4 and GP model followed them. Therefore, we recommended that APGA could be used instead of the GP model for the prediction significant fibrosis in CHB patients. However, further studies involving a greater number of patients and different populations are required to assess the applicability of this GP model.

REFERENCES