Staging of liver fibrosis in chronic hepatitis B patients with a composite predictive model: A comparative study

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Supported by Grant for Master Degree Students of Fudan University

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Received: October 28, 2009 Revised: December 9, 2009 Accepted: December 16, 2009 Published online: January 28, 2010

Abstract

AIM: To evaluate the efficacy of 6 noninvasive liver fibrosis models and to identify the most valuable model for the prediction of liver fibrosis stage in chronic hepatitis B (CHB) patients.

METHODS: Seventy-eight CHB patients were consecutively enrolled in this study. Liver biopsy was performed and blood serum was obtained at admission. Histological diagnosis was made according to the METAVIR system. Significant fibrosis was defined as stage score ≥ 2, severe fibrosis as stage score ≥ 3. The diagnostic accuracy of 6 noninvasive liver fibrosis models, including serum aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4, Forn’s index, Fibrometer, Hepascore, and Shanghai Liver Fibrosis Group’s index (SLFG), was investigated.

RESULTS: The APRI, FIB-4 and Forn’s index under receiver operating characteristic curve (AUROC) for significant fibrosis were 0.71, 0.75 and 0.79, respectively, with a diagnosis accuracy of 67%, 77% and 80%, respectively, and 0.80, 0.87 and 0.86, respectively, under the AUROC for severe fibrosis. The Hepascore, SLFG, and Fibrometer were 0.80, 0.83 and 0.85, respectively under the AUROC for significant fibrosis (P < 0.01). The diagnosis accuracy of Hepascore and SLFG was 86% and 88%, respectively. The Hepascore, SLFG, and Fibrometer were 0.95, 0.93, and 0.94, respectively, under the AUROC for severe fibrosis (P < 0.01).

CONCLUSION: The models containing direct serum markers have a better diagnostic value than those not containing direct serum markers.

Key words: Chronic hepatitis B; Liver fibrosis; Serum marker; Noninvasive model; Receiver operating curve

Peer reviewers: Damiao Carlos Moraes Santos, DCM, PhD, Bio-Manguinhos, Fundación Oswaldo Cruz, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, 21040360, Brazil; Roberto J Carvalho-Filho, MD, PhD, Hepatitis Section, Division of Gastroenterology, Federal University of Sao Paulo, Rua Botucatu, 740, 2.o andar, Vila Clementino, State of Sao Paulo, 04023-060, Brazil


INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 million individuals worldwide. At least one million people chronically infected with HBV would die of chronic liver diseases each year[1]. Thus, it is important to prevent the progression of early liver fibrosis to
cirrhosis. Although liver biopsy is the gold standard for the assessment of fibrosis, it has several disadvantages, such as poor patient compliance, sampling error, limited usefulness for dynamic surveillance, and poor intra- and inter-observation concordance. Considering these limitations, noninvasive histology predictors are urgently needed.

Since single fibrosis surrogate cannot measure fibrosis, an alternative approach combined with a number of parameters can generate algorithms capable of evaluating fibrosis. A number of noninvasive models containing serum markers, such as serum aspartate aminotransferase (AST) to platelet ratio index (APRI), Fibrometer, Hepascore, Shanghai Liver Fibrosis Group's index (SLF-G) have been studied worldwide. Additionally, except for SLF-G, little has been known about the role of these models in predicting fibrosis stage of chronic hepatitis B (CHB) because most studies were performed in chronic hepatitis C (CHC). China has a high prevalence of CHB, and most hepatocellular carcinomas result from chronic HBV infection. Therefore, we carried out this study to identify the best practical noninvasive model of liver fibrosis in CHB.

**MATERIALS AND METHODS**

**Patients**

Seventy-eight consecutive eligible CHB patients who underwent a liver biopsy in March 2006-August 2008 at Zhongshan Hospital, Fudan University, Shanghai, China, were included in this study. Blood serum was collected and stored at -80°C for further test. Chronic HBV infection was diagnosed based on positive surface antigen of HBV (HBsAg) and fluctuated alanine aminotransferase (ALT). Exclusion criteria included chronic liver disease due to other causes or co-infection with hepatitis D, clinically overt cirrhosis, previous or concomitant anti-HBV therapy, alcohol consumption exceeding 20 g/d in men and exceeding 10 g/d in women. Data were retrospectively analyzed. The study protocol was approved by the Institutional Review Board in our hospital. Written informed consent was obtained from each patient.

**Liver histology and quantification of fibrosis**

Liver tissue was obtained by sono-guided percutaneous biopsy (Bard®, Magnum®, 18G, USA) and stained with hematoxylin-cosin-safran and Masson's trichrome. Fibrosis staging (F) and inflammatory activity (A) were decided according to the METAVIR system. Fibrosis staging was divided into F0-F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = perportal fibrosis with few septa, F3 = septal fibrosis with many septa, and F4 = cirrhosis). Inflammatory activity was divided into A0-A3 (A0 = no histologic necroinflammatory activity, A1 = minimal activity, A2 = moderate activity, A3 = severe activity). The activity was assessed by integrating the severity and intensity of piecemeal (periportal) and lobular necrosis. According to the American Association for the Study of Liver Disease Practice Guidelines, we defined significant fibrosis as METAVIR fibrosis with a score ≥ 2 (F2, 3, 4) and severe liver fibrosis as METAVIR fibrosis with a score ≥ 3 (F3, 4).

**Serum parameters**

Following parameters, including AST, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), bilirubin, total cholesterol, urea, prothrombin time (PT), prothrombin index (PI), hemoglobin and platelet count (PLT) were assayed. AST, ALT, GGT, bilirubin, total cholesterol, urea were tested with Hitachi 7600, Japan. PT and PI were tested with Sysmex CA7000, Japan. Hemoglobin and PLT was tested with Sysmex routine blood test pipeline, Japan. The reference value was 0-75 IU/L for ALT and AST. Serum α2-macroglobulin (A2M) (GenWay Biotech, San Diego, USA) and hyaluronic acid (HA) (Shanghai High Medical Biotech, Shanghai, China) concentrations were measured by enzyme linked immunosorbent assay.

APRI, FIB-4, Forn's index, SLF-G, Hepascore, and Fibrometer were detected according to the following formulas: APRI = [AST (/ULN)/PLT (10^9/L)] × 100, FIB-4 = [age (yr) × AST (U/L)]/{[PLT (10^9/L)] × (ALT (U/L)/2)}, Forn's index = 7.811 - 3.131 × ln [PLT (10^9/L)] + 0.781 × ln [GGT (U/L)] + 3.467 × ln [age (yr)] - 0.014 × [cholesterol (g/L)], SLF-G = 10 × e^0.731 × (1+c^0.5), Y = - 13.995 + 3.220 × lg [A2M (g/L)] + 3.096 × lg [age (yr)] + 2.254 × lg [GGT (U/L)] + 2.437 × lg [HA (ng/mL)], Hepascore = c^0.5 × (1+c^0.5), Y = - 4.185818 - [0.0049 × age (yr)] + [0.7464 × sex (male = 1, female = 0)] + [1.0039 × A2M (g/L)] + [0.0302 × HA (ng/mL)] + [0.0691 × TB (μmol/L)] - [0.0012 × GGT (U/L)]; Fibrometer = -0.007 × PLT (10^9/L) - 0.049 × PI (%) + 0.012 × AST (U/L) + 0.005 × A2M (g/L) + 0.021 × HA (ng/mL) - 0.270 × urea (mmol/L) + 0.027 × Age (yr) + 3.718.

**Statistical analysis**

Statistical analysis was performed using Spearman's two-tailed test and univariate analysis. P < 0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values (NPV and PPV) were calculated by using cutoffs according to the original studies. The overall diagnostic performance of scores was evaluated by area under ROC curves (AUROCs). We used DANA method, which was developed by Poynard et al., to adjust the observed AUROCs in our study. All the AUROCs were adjusted to a standard DANA of 2.5 using the formula: Adjusted AUROC (AdAUROC) = Observed AUROC + 0.1056 × (2.5 - Observed DANA). The AUROCs were compared with the method of Hanley-McNeil.

**RESULTS**

**Patient characteristics**

The mean age of the 78 patients (66 males, 12 females) was 32.6 ± 12.3 years. The mean length of liver biopsies was 18.2 ± 3.4 mm, and the liver specimen length was longer than 15 mm. Significant fibrosis was found in 32
patients (41.0%), severe fibrosis in 19 patients (24.4%), and early cirrhosis in 9 patients (11.5%), respectively. Main features of the patients are summarized in Table 1.

### Correlation between non-invasive model and fibrosis stage

METAVIR fibrosis stages were significantly correlated with APRI, FIB-4, Forn’s index, Fibrometer, Hepascore and SLFG. A better correlation was observed between Fibrometer ($r = 0.69$), SLFG ($r = 0.68$) and Hepascore ($r = 0.62$) ($P < 0.001$). The box-plots of fibrosis scores are shown in Figure 1. A correlation was also found between scores and histological activity, especially between SLFG ($r = 0.55$), Fibrometer ($r = 0.54$) and APRI ($r = 0.51$) ($P < 0.001$).

### Overall diagnostic performance of serum markers

The mean levels of AST, GGT, PT, PI, AαM and HA were higher in patients with F2-F4 fibrosis than in those with F0-F1 fibrosis ($P < 0.01$). The mean levels of hemoglobin, PLT and albumin were lower in patients with F2-F4 fibrosis than in those with F0-F1 fibrosis ($P < 0.01$). Multiple regression analysis showed that AαM and HA were the independent factors for significant fibrosis (AαM, OR = 5.36, 95% CI: 1.58-18.13, $P = 0.007$; HA, OR = 1.01, 95% CI: 1.00-1.02, $P = 0.007$). AUROC was used to evaluate the overall diagnostic performance of scores (Table 2).

The APRI, FIB-4, Forn’s index, Hepascore, SLFG and Fibrometer were 0.71, 0.75, 0.79, 0.80, 0.83, and 0.85 respectively under the AUROC for F0-F4 (Figure 2A), and 0.75, 0.79, 0.83, 0.84, 0.86, and 0.88 respectively under the adjusted AUROC for F0-F4 with DANA method. The AUROC for Fibrometer, SLFG and Hepascore was better than that for APRI and FIB-4 ($P < 0.01$).

The APRI, FIB-4, Forn’s index, Hepascore, SLFG and Fibrometer were 0.80, 0.87, 0.86, 0.95, 0.93, and 0.94 under the AUROC for F0-F4 (Figure 2B). The Hepascore, Fibrometer and SLFG levels were significantly higher than the APRI level under the AUROC for F0-F4 ($P < 0.01$).

### Sensitivity, specificity, positive and negative predictive values

NPV and PPV for the diagnosis of significant fibrosis are presented in Table 3. Cutoffs were chosen for each model as previously described. This analysis could not be performed for Fibrometer since no cutoff was provided in the study by Calès et al[10]. When different cutoffs were used for each model, the percentage of classifiable subjects was 45%-78%, with a diagnostic accuracy of

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**Table 1**  Main characteristics of patients studied

<table>
<thead>
<tr>
<th></th>
<th>Patients ($n = 78$)</th>
<th>F0F1 ($n = 46$)</th>
<th>F2F3F4 ($n = 32$)</th>
<th>$P$ value (F0F1 vs F2F3F4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, yr)</td>
<td>32.6 ± 12.3</td>
<td>29.6 ± 12.0</td>
<td>36.9 ± 11.4</td>
<td>0.009</td>
</tr>
<tr>
<td>Men (n, %)</td>
<td>66 (84.6)</td>
<td>38 (82.6)</td>
<td>28 (87.5)</td>
<td>0.113</td>
</tr>
<tr>
<td>CHB family history (n, %)</td>
<td>29 (37.2)</td>
<td>18 (39.1)</td>
<td>11 (34.4)</td>
<td>0.104</td>
</tr>
<tr>
<td>WBC (mean ± SD, 10^3/L)</td>
<td>5.3 ± 1.4</td>
<td>5.5 ± 1.2</td>
<td>4.9 ± 1.6</td>
<td>0.060</td>
</tr>
<tr>
<td>Hb (mean ± SD, g/L)</td>
<td>142.6 ± 15.7</td>
<td>145.6 ± 13.4</td>
<td>138.3 ± 17.8</td>
<td>0.044</td>
</tr>
<tr>
<td>PLT (mean ± SD, 10^9/L)</td>
<td>170.2 ± 51.5</td>
<td>185.9 ± 40.7</td>
<td>147.6 ± 57.3</td>
<td>0.002</td>
</tr>
<tr>
<td>TB [median (interquartile range), μmol/L]</td>
<td>15.4 (11.5-20.6)</td>
<td>14.7 (10.6-19.1)</td>
<td>16.7 (12.1-24.1)</td>
<td>0.087</td>
</tr>
<tr>
<td>CB [median (interquartile range), μmol/L]</td>
<td>5.7 (4.0-7.8)</td>
<td>5.5 (3.9-7.1)</td>
<td>6.4 (4.5-10)</td>
<td>0.057</td>
</tr>
<tr>
<td>ALT [interquartile median (range), U/L]</td>
<td>115 (55-241)</td>
<td>93.5 (52-240)</td>
<td>132 (76-263)</td>
<td>0.165</td>
</tr>
<tr>
<td>AST [interquartile median (range), U/L]</td>
<td>67.5 (38-121)</td>
<td>56 (30-95)</td>
<td>86.5 (41-152)</td>
<td>0.042</td>
</tr>
<tr>
<td>GGT [interquartile median (range), U/L]</td>
<td>52.5 (27-176)</td>
<td>36.5 (21.59)</td>
<td>66.5 (46-94)</td>
<td>0.006</td>
</tr>
<tr>
<td>Alb (mean ± SD, g/L)</td>
<td>42.4 ± 5.1</td>
<td>44.0 ± 4.8</td>
<td>40.2 ± 4.6</td>
<td>0.001</td>
</tr>
<tr>
<td>PT (mean ± SD, s)</td>
<td>12.0 ± 1.1</td>
<td>11.5 ± 0.9</td>
<td>12.6 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PI (mean ± SD, s)</td>
<td>1.00 ± 0.8</td>
<td>1.03 ± 0.07</td>
<td>0.95 ± 0.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TC (mean ± SD, mmol/L)</td>
<td>3.8 ± 0.8</td>
<td>3.8 ± 0.8</td>
<td>3.7 ± 0.9</td>
<td>0.135</td>
</tr>
<tr>
<td>HA [interquartile median (range), ng/mL]</td>
<td>125 (75-224)</td>
<td>88 (49-129)</td>
<td>167 (116-382)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>A2M (mean ± SD, mg/L)</td>
<td>2.96 ± 0.58</td>
<td>2.73 ± 0.48</td>
<td>3.28 ± 0.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lg HBV-DNA (mean ± SD, log10 copies/mL)</td>
<td>6.0 ± 1.9</td>
<td>5.9 ± 2.0</td>
<td>6.1 ± 2.1</td>
<td>0.314</td>
</tr>
<tr>
<td>HBeAg positive (n, %)</td>
<td>32 (40.5)</td>
<td>32 (40.5)</td>
<td>23 (71.9)</td>
<td>0.223</td>
</tr>
<tr>
<td>Liver specimen length (mean ± SD, mm)</td>
<td>18.2 ± 3.4</td>
<td>18.4 ± 3.3</td>
<td>17.9 ± 3.6</td>
<td>0.254</td>
</tr>
</tbody>
</table>

| METAVIR A stage (n, %) | | | | |
|---|---|---|---|
| A0 | 4 (5.1) | | |
| A1 | 41 (52.5) | | |
| A2 | 32 (41.1) | | |
| A3 | 1 (1.3) | | |
| METAVIR F stage (n, %) | | | |
| F0 | 13 (16.7) | | |
| F1 | 33 (42.3) | | |
| F2 | 13 (16.7) | | |
| F3 | 10 (12.8) | | |
| F4 | 9 (11.5) | | |

SD: Standard deviation; WBC: Leucocyte; Hb: Hemoglobin; TB: Total bilirubin; CB: Conjugated bilirubin; Alb: Albumin; TC: Total cholesterol.
67%-86% (Table 4). Significant fibrosis (F2-4) was predicted in 13%-32% of patients with their PPV ranged 62%-88%. Since lower cutoffs were originally described to rule out significant fibrosis, attention must be paid to NPV ranging 76%-85%. The best positive predictive value (PPV = 0.88) for significant fibrosis was observed when SLFG > 8.7.

**DISCUSSION**

Noninvasive models have been proposed for the assessment of liver fibrosis. The diagnostic performance of APRI, FIB-4, Forn's index, Fibrometer, Hepascore and SLFG was evaluated for the assessment of liver fibrosis in CHB patients. These models are mainly based on two kinds of serum markers, direct and indirect. Direct serum markers are directly linked to the modifications in extracellular matrix (ECM) metabolism. Indirect serum markers have no direct link with liver fibrosis but reflect liver dysfunction or other phenomena caused by fibrosis. We focused on the serum markers of fibrosis. The main end-point of our study was to evaluate the global diagnostic performance of models by comparing their AUROCs. Our study indicated that models containing
regression analysis in our study showed that HA and AaM were the independent factor for significant fibrosis and had a better diagnostic accuracy.

It has been reported that APRI under the AUROC for significant fibrosis is 0.76 (95% CI: 0.74-0.79) [23]. It has been shown that the accuracy of APRI under the AUROC for significant fibrosis is 0.63 and 0.72 in CHB patients [24,25]. Zhang et al. [26] showed that APRI has low diagnostic accuracy of liver fibrosis and APRI combined HA can achieve a better diagnostic accuracy of liver fibrosis. FIB-4 has a high diagnostic accuracy of severe fibrosis [27]. Mallet et al. [28] reported that FIB-4 is 0.81 under the AUROC for severe fibrosis. In our study, the FIB-4 was 0.87 under the AUROC for severe fibrosis. The reported Forn's index is 0.76 under the AUROC for significant fibrosis [29]. In our study, the Forn's index was 0.79 under the AUROC for significant fibrosis. The diagnostic value of APRI, FIB-4 and Forn's index was much lower in CHB patients than in CHC patients.

Calès et al. [30] have developed Fibrometer for the diagnosis of significant fibrosis and found that its diagnostic performance is stable in patients with different chronic liver diseases [31,32]. Hepascore has been used in diagnosis of significant and severe fibrosis [33]. In our study, the Hepascore was 0.80 and 0.95 under the AUROC for significant and severe fibrosis. SLFG is the first developed noninvasive liver fibrosis model which is consistent with the findings in our study. Multiple
direct serum markers (Fibrometer, SLFG, Hepascore) performed much more accurately than models containing only indirect serum markers (APRI, FIB-4, Forn's index).

Direct serum markers are useful for assessing the speed of liver fibrogenesis. HA, a component of ECM, is a glycosaminoglycan synthesized by hepatic stellate cells and degraded by liver sinusoidal cells [18]. AaM is a protease inhibitor with its concentration increased due to stellate cell activation and liver fibrosis [19]. Studies have demonstrated that HA and AaM levels are correlated with hepatic fibrosis in patients with CHB or CHC [11,20-22], which is consistent with the findings in our study. Multiple
Noninvasive liver fibrosis model

data. These results indicate that FibroMeter, SLFG and Hepascore can be used in diagnosis of liver fibrosis. However, these noninvasive models should be validated in a larger number of CHB patients.

Broadly speaking, no true noninvasive model could exactly reflect liver fibrosis. Transient elastography (fibroScan) is another noninvasive method to detect the mean liver stiffness for diagnosing fibrosis. However, it is expensive and may be limited in those with narrow intercostal spaces, morbid obesity or significant ascites. These noninvasive models can be used in clinical management of CHB by offering an attractive alternative to liver biopsy.

In our study, since the sample size was small, further study is needed before these models are used in clinical practice. Validating against not only histological stage scores but also digital image analysis and clinical outcomes may also be a better choice.

In conclusion, serologic models containing direct serum markers of Hepascore, SLFG, and FibroMeter have better diagnostic values in CHB patients than those containing only indirect serum markers of APRI, FIB-4, Forn’s index.

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