Role of hyaluronic acid, its degrading enzymes, degradation products, and ferritin in the assessment of fibrosis stage in Egyptian patients with chronic hepatitis C
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**Background/aims** Liver biopsy is considered a gold standard for fibrosis staging, but it has a high risk of morbidity. Therefore, there is an interest in developing noninvasive markers for the prediction of liver fibrosis stages.

**Methods** Hyaluronic acid, ferritin, N-acetyl-\(\beta\)-o-glucosaminidase, \(\beta\)-glucuronidase, glucosamine, aspartate transaminase, and alanine transaminase were assayed in 210 individuals with chronic hepatitis C infection. Statistical analysis was carried out by logistic regression and receiver-operating characteristic curves.

**Results** The best linear combination of only significant blood markers was used for the determination of the fibrosis discriminant score; score = \[1.64 \text{ (numerical constant)} - 0.002 \times \text{hyaluronic acid (pg/l)} - 2.68 \times \beta\text{-glucuronidase (\(\mu\text{mol/ml/min)})} - 0.026 \times \text{glucosamine (\(\mu\text{g/dl)})} - 0.001 \times \text{ferritin} - 0.033 \times \text{aspartate transaminase/alanine transaminase}\]. The selected fibrosis discriminant score function correctly classified 81\% of patients with severe liver fibrosis at a discriminant cut-off score = 0.55 (i.e. less than 0.55 indicated mild liver fibrosis and greater than 0.55 indicated severe liver fibrosis), with a sensitivity of 100\% and a specificity of 73\%.

**Conclusion** A simple fibrosis index can be useful to select hepatitis C virus-infected patients with a very low risk of significant fibrosis in whom the protocol of liver biopsies may be avoided. *Eur J Gastroenterol Hepatol* 25:69–76 2013, 25:69–76

**Keywords:** blood, ferritin, fibrosis, hepatitis C virus, hyaluronic acid, liver

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**Introduction**
Liver fibrosis is a noticeable disease and may progress to liver cirrhosis or hepatocellular carcinoma gradually in patients with the hepatitis C virus (HCV). Although the pathogenesis of HCV-infected fibrosis is poorly understood, liver fibrosis may be a response of repair when the liver is injured or inflamed [1]. Staging liver fibrosis is considered to be essential in the management of patients with chronic hepatitis C (CHC), because it provides prognostic information and, in many cases, aids therapeutic decisions [2].

Although liver biopsy represents the gold standard for the evaluation of the presence, type, and stage of liver fibrosis and characterization of necroinflammation, it is an invasive procedure with inherent risks. Thus, it cannot be performed frequently to monitor therapeutic outcomes [3].

Therefore, development of noninvasive tests that can accurately predict the initial disease stage and progression over time is a high priority and a growing medical need.

Liver fibrosis is defined as building up of excessive amount of extracellular matrix (ECM) in the liver parenchyma. During fibrosis, hepatic stellate cells (HSC) play important roles in the control of ECM synthesis and degradation in fibrotic livers [4].

Hyaluronic acid (HA), a major ECM component, is a nonsulfated glycosaminoglycan composed of repeating polymeric disaccharides \(\beta\)-glucuronic acid and \(N\)-acetyl-\(\beta\)-glucosamine linked by a glucuronidic-\(\beta\)(1-3) bond [5].

The accumulation of HA may have been a consequence of either an increase in HA synthesis or a decrease in its degradation. An increase in the level of HA in liver diseases is responsible for glucuronic acid and glucosamine products [6].

Many studies have reported elevation in serum concentrations of HA in patients with active hepatitis compared with those of normal individuals [7]. HA is considered to be a better marker than other markers such as the amino-terminal propeptide of type III procollagen with respect to the prediction of development of cirrhosis as well as the prediction of symptoms [8].

\(\beta\)-Glucuronidase hydrolyzes the glucuronide bond at the nonreducing terminal of glycosaminoglycan [9]. A correlation was observed between the serum \(\beta\)-glucuronidase activity and the degree of hepatocellular necrosis in the liver biopsy specimens [10].
The liver is believed to be one of the organs that contains the highest hyaluronidase activity; thus, it is reasonable to consider that liver injury induces changes therein [11]. In addition, hyaluronidase activities are elevated after liver injury, and measures of circulating hyaluronidase activity may be used to assess liver damage [12].

N-acetyl-β-D-glucosaminidase catalyzes the removal of the N-acetylglucosamine residue from the nonreducing end of the oligosaccharide chains of glycoconjugates including HA [13]. It was used as a marker of tissue remodeling as in inflammatory processes [14]. Serum ferritin has been shown to be a predictor of mortality in patients with end-stage liver disease, both before and after liver transplantation [15]. Moreover, it has been reported that elevated serum ferritin levels were independently associated with advanced liver fibrosis, hepatic steatosis, and poor response to interferon (IFN) therapy for patients with CHC [16].

In the present study, we assessed and compared the diagnostic accuracy of serum HA, glucosamine, β-glucuronidase, hyaluronidase, N-acetyl-β-D-glucosaminidase, and ferritin levels as biochemical markers of severe liver fibrosis in chronic HCV patients using receiver-operating characteristic (ROC) curves. We also developed and evaluated a sensitive and specific fibrosis discriminant score (FDS) on the basis of these blood markers to predict severe liver fibrosis.

Materials and methods

Patients

The present study was carried out on patients from Mansoura University Hospitals, Mansoura, Egypt. A total of 210 Egyptian patients newly diagnosed with positive for HCV were included. The HCV infection was diagnosed on the basis of biochemical, serologic, and histological criteria. None of the patients had a history of habitual alcohol consumption or hepatocellular carcinoma. Moreover, all individuals were positive for anti-HCV antibody and were negative for hepatitis A and B virus testing. All patients were negative for anti-HIV antibodies. There were 164 men and 45 women, median age 39 years (range, 33–49 years). Patients were classified according to the histopathology investigation of liver biopsies with the Metavir staging system [17].

Liver biopsy

Needle liver biopsy specimens (n = 210) were taken from all patients and examined by a pathologist unaware of the laboratory results. Biopsies were processed for diagnostic purposes. Biopsies were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 4μm thickness, and routinely stained with hematoxylin and eosin. The patients were classified pathologically into different stages of fibrosis and from F0 to F4 according to the Metavir staging system [17].

Blood sample

Venous blood samples were collected into sterile glass tubes within 2 weeks of liver biopsy. The blood samples were allowed to coagulate at room temperature for 30 min and centrifuged at 2000g for 10 min. Serum was separated, aliquoted, and stored at –70°C until assays.

Measurement of hyaluronic acid level

Serum HA concentrations were determined using a commercial solid phase enzyme-linked immunosorbent assay (Tigsun Diagnostic Co. Ltd, Beijing, China). The kit was based on a competitive method to test the HA content in human serum. It mainly includes HA calibration serum, a horseradish peroxidase–HA conjugate, a microplate coated with HA antibody, and a substrate. Conjugate-labeled antigen and unlabeled antigen (patient samples) compete for the limited binding position of the microplate to form an antigen-antibody complex. Optical density was read using a microtiter plate reader (Dynex Technologies GmbH, Denkendorf, Germany) at dual wavelength (450 nm with a wavelength correction set to 640 nm). The assay has a sensitivity of 0.38 pmol/ml, with no significant cross-reactivity with other factors.

Measurement of hyaluronidase activity

Hyaluronidase activity was measured according to the method of Hutterer [18], with slight modifications. It was based on the determination of the N-acetylglucosamine-reducing end groups released from hyaluronate by the enzyme.

In brief, HA potassium salt (Sigma-Aldrich chemical Co., St Louis, Missouri, USA) was used as a substrate and dissolved in acetic acetate buffer (0.1 mol/l, pH 3.9) to obtain a buffered substrate solution. The buffered substrate was incubated with serum as a source of enzymes for 18 h, and then sodium carbonate (0.5 N) was added; the system was boiled to inhibit the enzymatic reaction. The mixture was cooled and glacial acetic acid and p-dimethyl amino benzaldehyde were added to the system. The absorption of the colored solution was measured at 583 nm. The amount of N-acetylglucosamine that was equivalent to the enzyme activity was obtained from the predesigned curve.

Measurement of N-acetyl-β-D-glucosaminidase activity

Serum N-acetyl-β-D-glucosaminidase activity was determined according to the method of Pugh et al. [19], with slight modifications. In brief, p-nitrophenyl-N-acetyl-β-D-glucosaminide was used as a substrate and acetic acid in sodium hydroxide (0.05 mol/l) was used as an acid buffer solution. A substrate buffer solution was freshly

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prepared and incubated with serum as a source of enzyme for an hour. After incubation, the enzymatic reaction was inhibited with sodium carbonate (3 N) and the color of the produced p-nitrophenol was calorimetrically measured at 410 nm.

**Measurement of β-glucuronidase activity**

β-Glucuronidase activity was assayed using the method of Goldbarg et al. [20], which is based on the conversion of p-nitrophenyl-β-D-glucuronic acid into p-nitrophenol and β-D-glucuronic acid by the activity of β-glucuronidase. In brief, sodium acetate (100 mmol/l, pH 5.0) was used as a buffer. The substrate was incubated with serum as a source of enzyme for 3 h. After incubation, the enzymatic reaction was stopped by the addition of sodium hydroxide (0.25 mol/l) and the color of the produced p-nitrophenol was calorimetrically measured at 410 nm.

**Measurement of glucosamine level**

Serum glucosamine level was assayed according to the methods of Boas [21], with slight modifications. This method was based on the fact that glucosamine hydrochloride was found to react with acetylacetone in an alkaline medium to form a product producing a red color with p-dimethyl-aminobenzaldehyde.

**Measurement of ferritin**

Serum ferritin was assayed using a solid-phase enzyme-linked immunosorbent assay kit (GenWay Biotech Inc., San Diego, California, USA). The assay system utilizes one rabbit anti-ferritin antibody for solid-phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody–enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After a 45-min incubation at room temperature, the wells were washed with water to remove unbound-labeled antibodies. A solution of tetramethylbenzidine reagent was added and incubated at room temperature for 20 min, resulting in the development of a blue color. The color development was stopped with the addition of stop solution, and the color changed to yellow and was measured spectrophotometrically at 450 nm.

**Statistical analysis**

All statistical analyses were carried out using the statistical software package ‘Medcalc version 11.3.3.0’ (Medcalc Software bvba, Mariakerke, Belgium) for Microsoft Windows and considered statistically significant at a two-sided P less than 0.05. Numerical data were expressed as mean±SD. The levels of markers were analyzed by analysis of variance, but the Mann–Whitney U-test was used for comparisons between independent groups. Correlation coefficients were calculated to assess the relationship between the histological degree of liver fibrosis and the concentration of serum markers. To assess the diagnostic accuracy of markers for the differentiation of chronic HCV patients, those with severe liver fibrosis from those with mild fibrosis, we plotted ROC curves [22] and the area under the curve (AUC) was calculated for comparison. An AUC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value [23]. The multivariate discriminant analysis (MDA) was carried out stepwise using minimum Wilk’s λ. The diagnostic sensitivity, specificity, efficiency, and positive predictive and negative predictive values were calculated.

**Results**

**Patient's characteristics**

According to histopathology examination for liver biopsies, our patients can be classified by a Metavir score into 90 patients (43%) with mild fibrosis (F1–F2) and 120 patients (57%) with severe fibrosis. In an attempt to identify biochemical analytes that could enhance the differentiation between patients with severe liver fibrosis (F3) and those with mild fibrosis (F1–F2), we measured eight analytes including HA, hyaluronidase, β-glucuronidase, N-acetyl-β-D-glucosaminidase, glucosamine, ferritin, aspartate transaminase (AST), alanine transaminase (ALT).

**Serum hyaluronic acid level, hyaluronidase, N-acetyl-β-D-glucosaminidase and β-glucuronidase activities**

As shown in Table 1, the serum HA level was significantly (P < 0.0001) elevated in patients with severe fibrosis (91.24±31.3 pg/l) (F1–F2) compared with those with mild liver fibrosis (60.31±29.31 pg/l). In addition, serum hyaluronidase activities were elevated in patients with mild fibrosis (60.99±22.89 N-acetyl-β-D-glucosaminidase/ml/18h) compared with that in patients with severe fibrosis (56.53±18.94 N-acetyl-β-D-glucosaminidase/ml/18h), but this elevation does not reach a statistically significant level (P = 0.23). Also, serum activities of N-acetyl-β-D-glucosaminidase were elevated in patients with mild fibrosis (19.06±8.09 μmol/ml/ min) compared with the activities in patients with severe fibrosis (18.45±10.23 μmol/ ml/min) and did not reach a statistically significant level (P = 0.674).

Table 1 shows that, there was a statistically significant (P = 0.0001) elevation in the mean serum activity of β-glucuronidase in patients with mild fibrosis (0.054±0.021 μmol/ml/min) compared with that in patients with severe fibrosis (0.039±0.024 μmol/ml/min).

**Serum glucosamine, ferritin, and aspartate transaminase/alanine transaminase ratio**

The serum level of glucosamine was significantly (P < 0.0001) elevated in patients with mild liver fibrosis (19.72±2.35 μg/dl) compared with patients with severe liver fibrosis (13.93±5.65 μg/dl) (Table 2). Also, Table 2 shows that the serum ferritin level was significantly (P < 0.0001) elevated in patients with severe fibrosis.
(154.95 ± 60.13 ng/ml) compared with patients with mild fibrosis (116.64 ± 72.59 ng/ml). The AST/ALT ratio was calculated for all patients and it was significantly (P < 0.0001) elevated in patients with severe liver fibrosis (1.24 ± 0.81) compared with that in patients with mild liver fibrosis (0.86 ± 0.31) (Table 2).

Table 1  Serum hyaluronic acid and its degradation enzymes involved hyaluronidase, N-acetyl-β-D-glucosaminidase, and β-glucuronidase in patients with mild and severe liver fibrosis

<table>
<thead>
<tr>
<th></th>
<th>Hyaluronic acid (pg/l)</th>
<th>Hyaluronidase (mg N-acetyl-β-D-glucosaminidase/ml/18 h)</th>
<th>N-acetyl-β-D-glucosaminidase (μmol/ml/min)</th>
<th>β-Glucuronidase (μmol/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild fibrosis (F1–F2)</td>
<td>60.31±29.31</td>
<td>60.99±22.89</td>
<td>19.06±0.09</td>
<td>0.054±0.021</td>
</tr>
<tr>
<td>Severe fibrosis (F3–F4)</td>
<td>91.24±31.3*</td>
<td>154.95±60.13</td>
<td>18.45±10.23*</td>
<td>0.039±0.024*</td>
</tr>
</tbody>
</table>

*P<0.0001; †P=0.0001; ‡P=0.23; ‡P=0.674.

Table 2  Serum glucosamine, ferritin, and AST/ALT ratio in patients with mild and severe liver fibrosis

<table>
<thead>
<tr>
<th></th>
<th>Glucosamine (μg/dl)</th>
<th>Ferritin (ng/ml)</th>
<th>AST/ALT ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild fibrosis (F1–F2)</td>
<td>19.72±2.35</td>
<td>116.64±72.59</td>
<td>0.86±0.31</td>
</tr>
<tr>
<td>Severe fibrosis (F3–F4)</td>
<td>13.93±5.65*</td>
<td>154.95±60.13*</td>
<td>1.24±0.81*</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase; AST, aspartate transaminase. *P<0.0001.

ROC curves were used to determine the cut-off values with the best efficiency of serum HA (83.5 pg/l), β-glucuronidase (0.063 μmol/ml/min), and glucosamine (12.5 μg/dl). Their AUCs were 0.647, 0.686, and 0.555, respectively (Fig. 1). Serum HA was the best efficient index. Therefore, we used serum HA as the basic index to combine with other indices to differentiate between severe liver and mild fibrosis.

Performance characteristics of the fibrosis discriminant score

The best linear combination of blood markers that were selected by the MDA was used to determine the fibrosis discriminant score (FDS) equation based on three markers (HA, β-glucuronidase, and glucosamine). Therefore, in an attempt to enhance the discriminating power of FDS, we included ferritin as a fourth marker (HA, β-glucuronidase, glucosamine, and ferritin) and then AST/ALT as a fifth marker (HA, β-glucuronidase, glucosamine, ferritin, and AST/ALT) to differentiate patients with severe liver fibrosis from those with mild liver fibrosis. The areas under the ROC curves of ferritin and AST/ALT were 0.68 and 0.747, respectively (Fig. 2). MDA selected a score on the basis of the absolute values of the five biochemical markers: FDS = [1.64 (numerical constant) − 0.002/C2 hyaluronic acid (pg/l) − 2.68/C2 β-glucuronidase (μmol/ml/min) − 0.026/C2 glucosamine (μg/dl) − 0.001/C2 ferritin − 0.033 (ng/ml) / AST/ALT] was selected (Table 3).

To evaluate the differential diagnostic power of the discriminant function, we constructed ROC curves for FDS and compared them with ROC curves of the other variables that we had previously found to significantly differentiate between severe and mild liver fibrosis. FDS was calculated for each patient on the basis of the linear combination of variables selected by MDA and used to classify cases into one of the two groups (mild and severe liver fibrosis). The areas under curves were 0.647, 0.686, and 0.555, respectively. ROC, receiver-operating characteristic.

ROC curves of a single measurement of hyaluronic acid, β-glucuronidase, and glucosamine for differentiating patients with severe liver fibrosis from patients with mild liver fibrosis. The areas under curves were 0.647, 0.686, and 0.555, respectively. ROC, receiver-operating characteristic.
Figure 3 shows the box plots for FDS in patients with mild liver fibrosis (F1–F2) and those who had (F3) severe liver fibrosis. The median, mean, and ±SD of FDS were 0.865, 0.869, and ±0.123 in patients with severe liver fibrosis and 0.657, 0.709, and ±0.194, respectively, in patients with mild fibrosis. When comparing the value of FDS in severe liver fibrosis and mild liver fibrosis, it was highly significant ($P < 0.0001$). AUCs were 0.735, 0.741, and 0.791 for three, four, and five markers, respectively (Fig. 4).

FDS correctly classified 81% of the patients with severe liver fibrosis (F1–F2) and those who had (F3) severe liver fibrosis. The median, mean, and ±SD of FDS were 0.865, 0.869, and ±0.123 in patients with severe liver fibrosis and 0.657, 0.709, and ±0.194, respectively, in patients with mild fibrosis. When comparing the value of FDS in severe liver fibrosis and mild liver fibrosis, it was highly significant ($P < 0.0001$). AUCs were 0.735, 0.741, and 0.791 for three, four, and five markers, respectively (Fig. 4).

Table 3  Multiple logistic regression model for FDS on the basis of absolute values of five blood markers (hyaluronic acid, $\beta$-glucuronidase, glucosamine, ferritin, and AST/ALT) for differentiating patients with severe liver fibrosis (F3) from those with mild liver fibrosis (F1–F2)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>SE</th>
<th>AUC</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>−0.002</td>
<td>0.102</td>
<td>0.647</td>
<td>0.447 – 0.847</td>
</tr>
<tr>
<td>$\beta$-Glucuronidase</td>
<td>−2.680</td>
<td>0.089</td>
<td>0.686</td>
<td>0.510 – 0.862</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>−0.026</td>
<td>0.137</td>
<td>0.555</td>
<td>0.229 – 0.766</td>
</tr>
<tr>
<td>Ferritin</td>
<td>−0.001</td>
<td>0.066</td>
<td>0.680</td>
<td>0.550 – 0.811</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>−0.033</td>
<td>0.061</td>
<td>0.734</td>
<td>0.613 – 0.855</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase; AST, aspartate transaminase; AUC, area under curve; CI, confidence interval; FDS, fibrosis discriminate score.

Figure 3 shows the box plots for FDS in patients with mild liver fibrosis (F1–F2) and those who had (F3) severe liver fibrosis. The median, mean, and ±SD of FDS were 0.865, 0.869, and ±0.123 in patients with severe liver fibrosis and 0.657, 0.709, and ±0.194, respectively, in patients with mild fibrosis. When comparing the value of FDS in severe liver fibrosis and mild liver fibrosis, it was highly significant ($P < 0.0001$). AUCs were 0.735, 0.741, and 0.791 for three, four, and five markers, respectively (Fig. 4).

FDS correctly classified 81% of the patients with severe liver fibrosis at a discriminant cut-off score = 0.55 [i.e. less than 0.55 indicated mild fibrosis (F1–F2)], and a score greater than 0.55 indicated severe liver fibrosis with a sensitivity of 100% and a specificity of 73%; the positive and negative values were 91 and 97%, respectively (Table 4).
In chronic hepatitis, HA is synthesized by HSCs and is metabolized in the liver endothelial cells [36]. With severe fibrosis, increasing deposition of basement membrane components caused capillarization of sinusoidal endothelial cells, therefore reducing this acid clearance. Therefore, the increase in the mean levels of HA in the sera of the patients of this study, especially in the sera of those with severe fibrosis, was expected and indicates the abnormalities in endothelial cells in the liver of such patients [37]. This capillarization of endothelial cells is correlated with the dynamicity of liver disease, increase in the levels of HA, and the severity of the disease. These data confirm the usefulness of HA measurements in the process of liver fibrogenesis. The increase in serum N-acetyl-β-D-glucosaminidase activity in fibrotic patients is attributable to the increased accumulation of ECM, including HA, typical criteria of the liver disease [38].

The increase in the activity of N-acetyl-β-D-glucosaminidase in the sera of our patients, especially those with early liver fibrosis, was expected. This is because HA levels were found to be increased in the sera of the patients of this study and this was potentiated with the severity of disease. Unfortunately, the latter increase in N-acetyl-β-D-glucosaminidase activity did not reach a statistically significant level \( (P = 0.23) \); thus, it was excluded from the construction of our MDA function. Moreover, the mean hyaluronidase activities was increased to share in the degradation of the accumulated HA and also did not reach the expected statistically significant level \( (P = 0.674) \); therefore, it was also excluded from the MDA function. It has been reported that β-glucuronidase activities is elevated in the livers of rats that were administered carbon tetrachloride to induce liver fibrosis [39,40]. The latter authors reported that the increase in the activities of that enzyme during induction of fibrosis was because of an elevation of its synthesis in the hepatocytes, the predominant cell type in the liver [39,40]. This increase may participate in the further degradation steps of HA. The increase in the activities of β-glucuronidase indicates hepatocellular necrosis, cell release, apoptosis, and the subsequent release β-glucuronidase into the bloodstream during the fibrogenesis process [39]. One cannot neglect the involvement of the latter mechanism in the elevation of β-glucuronidase during liver fibrogenesis. This is because the latter enzyme activity was significantly increased \( (P = 0.0001) \) in the sera of our patients.

Elevated iron-related serum markers and increased hepatic iron deposition are relatively common and correlate with the severity of hepatic inflammation and fibrosis in patients with CHC. In addition, iron accumulation in HCV-related chronic hepatitis is clinically relevant in hepatic damage and in hepatocytic oxidatively generated DNA damage, which was correlated with hepatic inflammation [41]. Therefore, one of these markers (ferritin) was included in MDA of this study.
An increased concentration of serum ferritin was observed in the sera of patients with liver fibrosis in our study, especially in the advanced stages of this disease, a finding that was expected. This is because an iron overload was found in the sera of patients with liver cirrhosis [42]. The results of ferritin in the present study together with that of Moon et al. [43] lead one to predict that our patients may suffer from an iron overload. The latter may induce oxidative stress through a Fenton-like reaction, thus participating in the development of hepatic fibrosis. Our results confirm those of Moon et al. [43], who evaluated the association between serum ferritin and hepatic iron deposition in hepatic fibrosis or inflammation and found that the ferritin levels were elevated and correlated with the degree of inflammation and fibrosis.

Similarly, Griffiths et al. [44] observed that elevation in iron may promote liver injury and inflammation in CHC infection and decrease the response to IFN therapy. Recently, Fujita et al. [45] observed two iron-related protein peaks, namely, hemopexin and transferring. These bands were found in the sera of patients with severe liver fibrosis. Another explanation for the increase in serum ferritin during fibrogenesis is based on the ability of the hepatocyte to secrete it. Also, it has been observed that ferritin excretion promotes Fas-mediated apoptosis [46]. Interestingly, a recent study has shown that ferritin can act as a proinflammatory cytokine in activated HSCs, in an iron-independent manner, through induction of a signaling cascade involving phosphoinositide 3-kinase, mitogen-activated protein extracellular signal-related kinase 1/2, and mitogen-activated protein kinase [47]. In addition, serum ferritin has been shown to be a predictor of mortality in patients with end-stage liver disease, both before and after liver transplantation [15,48]. The latter processes may be involved in the activation of HSCs, thus inducing ECM remodeling including overproduction of HA. Therefore, ferritin was included in our MDA for enhancing the diagnostic accuracy of the function and it was significantly elevated in patients with severe fibrosis (AUC = 0.680).

Tangkijvanich et al. [49] identified a cut-off point that differentiated between liver cirrhosis, chronic hepatitis, hepatocellular carcinoma, and healthy controls with a sensitivity, specificity, and accuracy of 82.4, 78.2, and 80.2%, respectively. Lackner et al. [50] validated and compared the diagnostic accuracies of the simple fibro tests including the cirrhosis discriminant score, the AST-to-platelets ratio index, and the platelet count. ROC curves of these simple fibro tests showed comparable diagnostic accuracies for the prediction of significant fibrosis (0.71, 0.74, and 0.80, respectively).

The ROC curves in our study showed that no single serum index had an ideal AUC; however, serum HA was the most efficient index among other markers with AUC (0.697). Therefore, the MDA function based on three biochemical markers (HA, β-glucuronidase, and glucosamine) showed an AUC of 0.735 and that of four biochemical markers (HA, β-glucuronidase, glucosamine, and ferritin) was 0.741. In addition, AUC of five biochemical markers (HA, β-glucuronidase, glucosamine, ferritin, and AST/ALT ratio) showed an AUC of 0.791, and was chosen to differentiate patients with severe liver fibrosis from those with mild liver fibrogenesis. The AST/ALT ratio was included in the MDA score because it has shown good diagnostic accuracy in patients with chronic viral liver disease [51]. The diagnostic value of five laboratory markers was assessed using logistic regression analysis, and the score was constructed by combining the most significant factors identified.

Although the AUC of MDA (FDS) score in our results was lower than that obtained by Hepascore [52], which was based on bilirubin, γ-glutamyltransferase, HA, α(2)-macroglobulin, age, and sex, our score was based on HA, which is considered the main backbone of GAGs and one of the major components of ECM that reflects the dynamic state of the actual process of liver fibrogenesis. Our FDS function correctly classified 81% of severe fibrosis at a discriminant cut-off score = 0.55 [i.e. less than 0.55 indicated patients with mild liver fibrosis (F1–F2) and greater than 0.55 indicated patients with severe liver fibrosis] with the highest degrees of sensitivity (100%) and specificity (73%). The positive predictive and negative predictive values were also high (91 and 97%, respectively).

The sensitivity and specificity of our combinations were higher than those in other reports [51]. Hence, this FDS allowed us to accurately predict the presence of severe liver fibrosis in patients infected with HCV. The area under the ROC of our combination was similar to that of other reports [53].

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

References