Non-invasive Assessment of Liver Fibrosis Using Serum Markers

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ABSTRACT

Fibrosis is an intrinsic response to chronic injury, that elicits excess deposition of matrix compounds and scarring, which ultimately alters liver structure and function. Liver biopsy has long been the gold standard for assessing the degree of liver fibrosis. Due to liver biopsy invasiveness, specialists and patients favor alternative non-invasive tests for fibrosis staging. In recent years, many non-invasive tests based on measurement of direct or indirect serum markers have been developed. Direct and indirect markers may be used alone or, more commonly, in combination with each other, to produce composite liver fibrosis scores/panels. Almost all of them have the ability to identify significant fibrosis and cirrhosis in particular. However, only those tests with the highest diagnostic accuracy and availability should be introduced. Apart from their diagnostic accuracy, the potential ability of these tests to predict disease outcomes should be compared with that of liver biopsy. Continued research in this area will give the opportunity to offer patients with liver diseases more precise and non-invasive diagnostic tools. Until now liver biopsy still a part of clinical practice but we hope progress in biomedicine can change the current situation in the next few decades. Consequently, liver biopsy will be a history.

Keywords: Non-invasive; liver fibrosis; serum markers; scores; panels

INTRODUCTION

More than 2,000 studies in the last five years have employed serological markers to assess liver fibrosis [1]. Several non-invasive markers are currently being validated as potential tools to determine liver damage and some of them are now commercially available but they have not been yet incorporated in clinical practice in most countries [2]. An exception to this rule is France, where three well-validated non-invasive methods (Fibrotest, Fibrometer, and FibroScan) have been approved by the public health system and are routinely used in clinical practice [3].
Serum markers that reflect liver function (indirect markers) or that related to matrix metabolism (direct markers) or combinations of both are currently used. Recent studies have proposed multiple indices based on the combination of: (1) indirect and direct serum markers (e.g. SHASTA index), (2) serum markers with other different non-invasive methods (e.g. PLF score), (3) serum biochemical markers with clinical data (e.g. Fib-4) or (4) serum biochemical and variable clinical markers with fibrosis parameters (e.g. Fibrometer) to increase the diagnostic accuracy [1, 4]. The best marker panels show the area under receiver operating characteristic curves (AUROCs) around 0.8–0.85 to differentiate between no/mild fibrosis (Metavir F0–F1) and moderate/severe fibrosis (F2–F4). Fibrosis markers have almost exclusively been validated as predictors of fibrosis stage, while especially the direct parameters may rather reflect the dynamics of fibrogenesis and/or fibrolysis [1].

1. Fibrosis
Fibrosis is the excess accumulation of extracellular matrix proteins (ECMP), which results from chronic inflammation. This inflammation triggers a wound-healing process that mitigates inflammatory tissue destruction but also leads to scar tissue formation.

2. Liver fibrosis history
Liver fibrosis results from chronic damage to the liver in conjunction with the accumulation of ECMP, which is a characteristic of most types of chronic liver diseases. The accumulation of ECMP distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension. Hepatic fibrosis was historically thought to be a passive and irreversible process. Currently, it is considered a model of the wound-healing response to chronic liver injury. Since the demonstration, in the 1990s, that even advanced liver fibrosis is reversible, researchers have been stimulated to identify antifibrotic therapies [5].

3. Liver fibrosis etiology and consequences

3.1 Etiology
Liver fibrosis is mainly due to chronic viral hepatitis B or C, autoimmune and biliary diseases, alcoholic steatohepatitis (ASH) and nonalcoholic steatohepatitis (NASH) (Figure 1). While mild fibrosis remains largely asymptomatic, its progression toward cirrhosis is the major cause of liver-related morbidity and mortality [1].

Fig. 1: The main liver fibrosis etiologies
NASH, Nonalcoholic steatohepatitis; HCC, Hepatocellular carcinoma.

3.2 Clinical consequences
(a) Fibrosis progression toward cirrhosis;
(b) Liver functional failure, including failing hemostatic, nitrogen handling, and detoxification systems;
(c) Portal hypertension with consequent formation of ascites and bleeding esophageal or gastric varices;
(d) High susceptibility to infection; and
(e) High risk to develop hepatocellular carcinoma (HCC)

4. Pathogenesis of liver fibrosis
Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury (Figure 2) [6]. After an acute liver injury, parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of ECM. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECMP. As fibrotic liver diseases advance, disease progression from collagen bands to bridging fibrosis to frank cirrhosis occurs [7]. In advanced stages, the liver contains approximately 6 times more ECM components than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM components results from both increased synthesis and decreased degradation. Decreased activity of ECM-removing matrix metalloproteinases (MMPs) is mainly due to an overexpression of their specific tissue inhibitors (TIMPs) [8].

![Diagram](attachment:image.png)

**Fig. 2: Changes in the hepatic architecture (A) associated with advanced hepatic fibrosis (B).**
Following chronic liver injury, inflammatory lymphocytes infiltrate the hepatic parenchyma. Some hepatocytes undergo apoptosis, and Kupffer cells activate, releasing fibrogenic mediators. Hepatic stellate cells (HSCs) proliferate and undergo a dramatic phenotypical activation, secreting large amounts of extracellular matrix proteins. Sinusoidal endothelial cells lose their fenestrations, and the tonic contraction of HSCs causes increased resistance to blood flow in the hepatic sinusoid. Figure modified with permission from Science & Medicine (S28).
5. Liver fibrosis scoring/staging

5.1 Importance of liver fibrosis staging

Fibrosis stages assessment is important to determine the prognosis of chronic liver disease, select patients for specific treatment, and to monitor the success of treatment [9]. The history of the fibrosis scoring systems dates back to 1981 when the histological features of chronic hepatitis were evaluated for potential importance in determining its prognosis by Knodell and colleagues [10]. The Ishak score, or revised Knodell system, considers grading and staging as two separate items; liver fibrosis is classified as: 0 = absent, 1-2 = mild, 3-4 = moderate and 5-6 = severe/cirrhosis. The grading system has been subsequently modified by other pathologists. The Metavir classification system includes two separate scores, one for necroinflammatory grade (A) and another for the fibrosis stage (F). The grade is usually scored from 0-4, with A0 being no activity and A3 to A4 considered severe activity. The stage scored from F0 to F4 (Figure 3): F0 is the absence of fibrosis, F1 is portal fibrosis without septa, F2 is portal fibrosis with rare septa, F3 is numerous septa without cirrhosis, and F4 is cirrhosis [11].

The current modalities used for quantifying and staging hepatic fibrosis are summarized in Figure 4.

5.2 Liver fibrosis evaluation methods

They can be divided into:

(1) Invasive: For the past 50 years liver biopsy has been considered to be the gold standard for staging of liver fibrosis. This technique allows physicians to obtain diagnostic information not only on fibrosis, but also on many other liver injuring processes, such as inflammation, necrosis, steatosis, hepatic deposits of iron or copper [12]. However, many studies clearly highlight several crucial drawbacks of liver biopsy, including variable accessibility, high cost, sampling errors and inaccuracy due to inter- and intra-observer variability of pathologic interpretations [12]. In addition, there is a small but important risk of liver biopsy-associated morbidity and mortality; pain, hypotension, intraperitoneal bleeding and injury to the biliary system. The risk for hospitalization after liver biopsy is 1-5%, the risk for severe complications is 0.57%, and mortality rate is 0.009-0.12% [14, 15]. Because of these reasons, some patients may choose to go without liver biopsy.

(2) Non-invasive: It include: (1) serum tests, (2) proteomic profiles/genetic tests, and (3) physical (imaging) techniques. In addition to being less invasive, there is a low risk of sampling error and small observer-related variability. Moreover, measurements may be performed repeatedly over time, allowing for ongoing monitoring of fibrosis [16]. Most serum biomarkers have only been used as investigative, rather than diagnostic, parameters in the clinic [17].
Liver biopsy is an invasive method that remains an imperfect golden standard. Proteomics based profiles are unlikely to be introduced to routine clinical care anytime soon, but are valuable from the research point of view. Imaging techniques, serum biomarkers and biomarker panels are advancing along the route to the clinic stage, but require extensive validation [13].

5.3 Classification of liver fibrosis serum markers [18]

Direct serum markers based on ECMP reflecting the activity of the fibrotic process, and are thought to indicate the extent of connective tissue deposition. Indirect serum markers based on routine liver function parameters that have been used in clinical practice.

Direct and indirect markers may be used alone or, more commonly, in combination with each other, to produce composite scores/panels. The calculation of such scores can be relatively simple or can be based on complicated formulas [13].

6. Criteria for ideal non-invasive serum markers

The ideal non-invasive marker would have the following characteristics [13, 19]: simple, safe, readily available, inexpensive, accurate, reproducible, and sensitive to the effects of treatment, specific to identify different stages of fibrosis, useful in tracking disease progression or regression and not be susceptible to false positive results.

The diagnostic performances of fibrosis markers are assessed by receiver operating characteristic (ROC) curves. The most commonly used index of accuracy is the area under the ROC (AUROC) curve with
values close to 1 indicating a high diagnostic accuracy [20].

7. Direct serum fibrosis markers (Table 1)

Several fibrosis panels have been developed and some of them are now available for commercial use [9].

Table 1. Direct serum markers of liver fibrosis

<table>
<thead>
<tr>
<th>No.</th>
<th>Serum marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Transforming growth factor-β1 (TGF-β1)</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Procollagen type I carboxy-terminal peptide (PICP)</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>Procollagen type III amino-terminal peptide (PIIINP)</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Hyaluronic acid (HA)</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Matrix metalloproteinases (MMPs) [MMP-2, MMP-3 and MMP-9]</td>
<td>25-28</td>
</tr>
<tr>
<td>6</td>
<td>Tissue inhibitors of metalloproteinases (TIMPs) [TIMP-1, TIMP-2]</td>
<td>26, 28</td>
</tr>
<tr>
<td>7</td>
<td>YKL-40 (chondrex)</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>Connective tissue growth factor (CTGF)</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>Paraoxonase 1 (PON-1)</td>
<td>31</td>
</tr>
<tr>
<td>10</td>
<td>Laminin</td>
<td>33</td>
</tr>
<tr>
<td>11</td>
<td>Microfibril-associated glycoprotein 4 (MFAP-4)</td>
<td>34</td>
</tr>
</tbody>
</table>

7.1 Transforming growth factor-β1 (TGF-β1): It is a cytokine involved in tissue growth, differentiation, ECM components production and the immune response. TGF-β1 is commonly accepted as a central component of fibrogenic response to wounding and is up-regulated in a variety of different diseases. A correlation between TGF-β1 levels and the rate of fibrosis progression is widely accepted [13].

7.2 Extracellular matrix components

7.2.1 Procollagen peptides

Procollagen type I carboxy-terminal peptide (PICP) levels are normal in patients with mild chronic hepatitis C (CHC) and elevated in 50% of patients with moderately advanced or advanced chronic hepatitis C, including patients with liver cirrhosis of this etiology [21].

Procollagen type III amino-terminal peptide (PIIINP) is a product of cleavage of procollagen and has been proposed as a serum marker of hepatic fibrosis more than two decades ago [22]. Its relative concentration in the basement membrane is higher in hepatic fibrogenesis [23]. In several studies in patients with chronic HCV infection, this biomarker showed only moderate diagnostic accuracy [9]. Unfortunately, PIIINP is not specific for the fibrosis of the liver as it is also elevated in acromegaly, lung fibrosis, chronic pancreatitis, and rheumatologic disease [18].

7.2.2 Hyaluronic acid (HA): HA is a polysaccharide present in ECM and elevated in serum in patients with hepatic fibrosis. Commercial test kits are available from Corgenix (Westminster, Colorado). The diagnostic accuracy of HA was found to be superior to that of PIIINP [24].

7.2.3 Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs): Excess ECM are degraded by matrix metalloproteinases which are in turn inhibited by tissue inhibitors of metalloproteinases (TIMPs). The three most commonly studied MMPs are MMP-2, MMP-3, and MMP-9. MMP-2 is secreted by activated HSCs; elevated levels of MMP-2 and its proenzyme have been observed in various liver diseases [25]. During hepatic fibrogenesis, the expression of MMP-2 is markedly increased. The potential for MMP-2 for predicting liver fibrosis remains unclear as some contradictory data have been reported by studies performed so far [26, 27]. In one study, MMP-9 levels were negatively correlated to the histological severity of the liver disease in patients with CHC [28].

Tissue inhibitors of metalloproteinases: TIMP-1 controls activity of most MMPs, whereas TIMP-2 specifically inhibits MMP-2. TIMPs-dependent inhibition of ECM degradation may promote liver...
fibrosis; elevation of TIMPs’ levels has been observed in chronic liver disease. For example, CHC causes the elevation of both TIMP-1 and TIMP-2 in corollary with fibrosis progression [26]. A recent study showed that serum levels of MMP-9 in chronic hepatitis patients were low as compared to the controls. Moreover, serum MMP-9 levels decrease as chronic hepatitis progresses to cirrhosis, while TIMP-1 levels increase along with an increase of the degree of fibrosis. These findings prompt using serum TIMP-1 as a non-invasive assay in liver fibrosis [28]. Thus, TIMP-1 is a component of several composite fibrosis panels.

7.2.4 YKL-40 (chondrex): YKL-40 is a mammalian glycoprotein homologue of the bacterial chitinases involved in remodeling or degradation of the extracellular matrix [29]. In liver diseases, serum levels of YKL-40 are closely related to the degree of histologically documented fibrosis [30].

7.2.5 Connective tissue growth factor (CTGF): It is synthesized in response to profibrogenic factor TGF-β by both activated HSC and hepatocytes. However, serum CTGF levels decrease in the end-stage cirrhosis [18].

7.2.6 Paraoxonase 1 (PON-1): It is an enzyme that hydrolyzes lipid peroxides, has antioxidant properties and influences hepatic cell apoptosis. Measurement of serum PON-1 activity has been proposed as a potential test for the evaluation of liver function, however, its clinical acceptance is limited due to instability and toxicity of its substrate, paraoxon [31].

7.2.7 Laminin: It is a major non-collagenous glycoprotein synthesized by the HSC and deposited in the basement membrane of the liver. During fibrosis, laminin accumulates around the vessels, in the perisinusoidal spaces and near the portal tract [32]. Elevated levels of laminin and pepsin resistant laminin (laminin P1) were found to correlate with the degree of perisinusoidal fibrosis [33].

7.2.8 Microfibril-associated glycoprotein-4 (MFAP-4): It is a ligand for integrins. Quantitative analysis of MFAP-4 serum levels showed high diagnostic accuracy for the prediction of non-diseased liver versus cirrhosis (AUROC = 0.97) as well as stage 0 versus stage 4 fibrosis (AUROC = 0.84), and stages 0 to 3 versus stage 4 fibrosis (AUROC = 0.76) [34].

8. Indirect serum fibrosis markers (Table 2)
Most serum indirect indices composed of routine laboratory parameters that reflect changes in liver function are readily available at no additional cost.

<table>
<thead>
<tr>
<th>No.</th>
<th>Score/Index (Reference)</th>
<th>Calculation/Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AST/ALT ratio (AAR score) [35]</td>
<td>AST (IU/L)/ALT (IU/L)</td>
</tr>
<tr>
<td>2</td>
<td>Age-AST model [37]</td>
<td>Age-AST model = Age (year)/AST (IU/L)</td>
</tr>
<tr>
<td>3</td>
<td>Age-platelet index (API) [40]</td>
<td>API = Age (year)/PLT (× 10⁹/L)</td>
</tr>
<tr>
<td>4</td>
<td>Cirrhosis discriminant score (CDS) [41]</td>
<td>AST/ALT(IU/L), INR and platelet</td>
</tr>
<tr>
<td>5</td>
<td>AST to platelet ratio index (APRI) [42]</td>
<td>[(AST/Upper limit of normal)/platelet count (10⁹/L)] × 100</td>
</tr>
<tr>
<td>6</td>
<td>Globulin/platelet (GP) model [44]</td>
<td>GP model = GLOB (g/mL) × 100/PLT (× 10⁹/L)</td>
</tr>
<tr>
<td>7</td>
<td>Fibro-quotient (FibroQ) index [45]</td>
<td>FibroQ = 10 × (age × AST × INR)/(ALT × platelet count)</td>
</tr>
<tr>
<td>8</td>
<td>PGA index [46, 47]</td>
<td>Prothrombin Index, γ-glutamyl transferase levels and apolipoprotein A1</td>
</tr>
<tr>
<td></td>
<td><strong>Index/Model</strong></td>
<td><strong>Formula</strong></td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>9</td>
<td>Göteborg University Cirrhosis Index (GUCI) [48]</td>
<td>((\text{AST} \times \text{INR} \times 100)/\text{platelet count (10}^9/\text{L}))</td>
</tr>
<tr>
<td>10</td>
<td>King's score [49]</td>
<td>King's score = age (years) (\times) AST (IU/L) (\times) INR/platelet count (10(^9/\text{L}))</td>
</tr>
<tr>
<td>11</td>
<td>SHASTA index [50]</td>
<td>Serum hyaluronic acid (HA), AST, and albumin</td>
</tr>
<tr>
<td>12</td>
<td>Pohl score [51]</td>
<td>AST/ALT (IU/L) and platelet number/µL</td>
</tr>
<tr>
<td>13</td>
<td>Fibrosis 4 score (FIB-4) [43]</td>
<td>FIB-4 = age (years) (\times) AST (IU/L)/platelet count (10(^9/\text{L})) (\times) ALT (IU/L(^{1/2}))</td>
</tr>
<tr>
<td>14</td>
<td>Fibrosis-cirrhosis index (FCI) [53]</td>
<td>FCI = (ALP \times \text{bilirubin})/(albumin \times \text{platelet count})</td>
</tr>
<tr>
<td>15</td>
<td>Forns index (FORNS) [54]</td>
<td>7.811 - 3.131 (\times) ln (platelet count (10(^9/\text{L}))) + 0.781 (\times) ln (GGT) + 3.467 (\times) ln (age) - 0.014 (\times) (cholesterol [mg/dL])</td>
</tr>
<tr>
<td>16</td>
<td>LOK (Model 3) [56]</td>
<td>Log odds = -5.56 - 0.0089 (\times) platelet (× 10(^9/\text{L})) + 1.26 (\times) AST/ALT ratio + 5.27 (\times) INR</td>
</tr>
<tr>
<td>17</td>
<td>Fibrotest (Fibrosure) [57]</td>
<td>(\alpha_2)-macroglobulin, haptoglobin, apolipoprotein A1, GGT, and total bilirubin</td>
</tr>
<tr>
<td>18</td>
<td>FibroIndex [58]</td>
<td>1.738 - 0.064 (platelets (\times 10^4/\text{mm}^3)) + 0.005 (AST [IU/L]) + 0.463 (gamma globulin [g/dL])</td>
</tr>
<tr>
<td>19</td>
<td>Fibrometer test* [59]</td>
<td>(\alpha_2)-macroglobulin, HA, AST, platelet count, prothrombin index, urea, and age</td>
</tr>
<tr>
<td>20</td>
<td>Fibrospect II assay* [61]</td>
<td>HA, TIMP-1 and (\alpha_2)-macroglobulin</td>
</tr>
<tr>
<td>21</td>
<td>Enhanced liver fibrosis (ELF) (ELFTM test)* [62]</td>
<td>Age, HA, PIIINP, and TIMP-1</td>
</tr>
<tr>
<td>22</td>
<td>Hepascore [63]</td>
<td>Bilirubin, GGT, HA, (\alpha_2)-macroglobulin, age and sex</td>
</tr>
<tr>
<td>23</td>
<td>TGF-(\beta_1), HA, PIIINP and TIMP-1 panel [64]</td>
<td>TGF-(\beta_1), HA, PIIINP and TIMP-1</td>
</tr>
<tr>
<td>24</td>
<td>Fibroscan/Fibrotest [65]</td>
<td>Fibroscan and Fibrotest</td>
</tr>
<tr>
<td>25</td>
<td>Sequential algorithms [66]</td>
<td>APRI, Fibrotest and/or Forns index</td>
</tr>
<tr>
<td>26</td>
<td>Predicted liver fibrosis score (PLF score) [67]</td>
<td>Transient elastography (TE) (Fibroscan) and multiple serological tests [PLF score = 0.956 + 0.084 (\times) TE - 0.004 (\times) King score + 0.124 (\times) Forns score + 0.202 (\times) APRI score]</td>
</tr>
</tbody>
</table>

INR, prothrombin time expressed as international normalized ratio; PLT, platelet; TIMP-1, tissue inhibitor of metalloprotease-1; PIIINP, procollagen type IIIa terminal peptide; TGF-\(\beta_1\), transforming growth factor \(\beta_1\). *Means the test is commercially available

8.1 Aspartate aminotransferase/Alanine aminotransferase ratio (AAR score) [35]: It is calculated as: AAR score = AST (IU/L)/ALT (IU/L). In a study of Ansar et al. (2009) the mean AAR value was found to be higher for each increasing stage of fibrosis. This finding is related to an increased release of mitochondrial AST, decreased AST clearance and/or impaired synthesis of ALT in advanced liver disease [36].

8.2 Age-AST model [37]: It is calculated as: Age-AST model = Age (year)/AST (IU/L).

8.3 Platelet count: Hepatic fibrosis may lead to thrombocytopenia as a consequence of impaired synthesis of thrombopoietin and/or sequestering of
platelets in an enlarged spleen. Surprisingly, few data exist on the diagnostic value of platelet count per se although the platelet count has been included in several composite fibrosis scores. Ono et al. (1999) reported the use of platelet count could discriminate F4 from F1-F3 in 75%-80% of patients with CHC [38]. In Lackner et al. (2005) study, a platelet count of < 150 × 10^9/L had a positive predictive value (PPV) > 90% for significant fibrosis, whereas at a cut-off of ≥ 150 × 10^9/L it had a negative predictive value (NPV) > 90% for cirrhosis [39].

8.4 Age-platelet index (API) [40]: It is calculated as API = Age (year)/PLT (× 10^9/L) [37].

8.5 Cirrhosis discriminant score (CDS): Platelet count has been also combined with AAR and prothrombin time in the CDS [41] but the diagnostic accuracy of these composite scores was not superior to platelet count per se [39].

8.6 AST to platelet ratio index (APRI) [42]: It is calculated as: APRI = [(AST/Upper limit of normal)/platelet count (10^9/L)] × 100. Its diagnostic accuracy for both significant fibrosis and cirrhosis has been confirmed. Using the cut-offs proposed by Wai et al. [42], approximately 50% of the patients can be correctly classified without a liver biopsy. In recent study, Mean APRI values significantly increased with successive fibrosis levels and the AUROC distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) was 0.8 [43].

8.7 Globulin/platelet (GP) model: It is calculated as: GP model = GLOB (g/mL) × 100/PLT (× 10^9/L). Using this model chronic HBV-infected patients with minimal fibrosis and cirrhosis can be diagnosed accurately, and the clinical application of this model may reduce the need for liver biopsy in HBVinfected patients [44].

8.8 Fibro-quotient (FibroQ) index: It includes prothrombin time international normalized ratio (INR), platelet count, AST, ALT, and age, and it is calculated as FibroQ = 10 × (age × AST ÷ INR)/(ALT × platelet count) to predict significant fibrosis [45].

8.9 PGA index: It combines the measurement of the prothrombin index, γ-glutamyl transferase levels and apolipoprotein A1. It was subsequently modified to the PGAA index by the addition of α2-macroglobulin, which resulted in marginal improvement in its performance. However, overall accuracy of this index is relatively low [46, 47].

8.10 Göteborg University Cirrhosis Index (GUCI): GUCI = (ASTxINRx100)/platelet count (10^9/L). It can with a high degree of accuracy discriminate patients with from those without hepatitis C-related significant fibrosis and cirrhosis [48].

8.11 King’s score: It is calculated as: King’s score = age (years)xAST (IU/L)xINR/platelet count (10^9/L). It is a simple and accurate index for predicting cirrhosis in chronic hepatitis C. The AUROC for predicting cirrhosis and significant fibrosis (F3-6) were 0.91 and 0.79, respectively [49].

8.12 SHAsta index: It consists of serum HA, AST, and albumin was evaluated in 95 patients with HIV/HCV co-infection. It has performed significantly better than the APRI test [50].

8.13 Pohl score: It includes AST/ALT(IU/L) and platelet number/µL. In patients with hepatitis C with AST/ALT less than 1 and platelets less than 150,000 are excluded from marked fibrosis [51].

8.14 Fibrosis 4 score (FIB-4): It is calculated as: FIB-4 = age (years)xAST (IU/L)/platelet count (10^9/L)xALT (IU/L)^1/2. Holmberg et al. (2013) reported that, mean FIB-4 values significantly increased with successive fibrosis levels and the AUROC analysis distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) was 0.83 [43]. Recently, Li et al. (2014) expanded and validated serum marker indices of fibrosis assessment in 284 chronic hepatitis B (CHB) and 2304 CHC patients. For the prediction of advanced fibrosis and cirrhosis, the FIB-4 score outperformed the other serum marker indices in the CHC cohort and was similar to APRI in the CHB cohort. The AUROC for FIB-4 in differentiating F3-F4 from F0-F2 was 0.86 for CHB and 0.83 for CHC [52].
8.15 Fibrosis-cirrhosis index (FCI): FCI = (ALPxbilirubin)/(albuminxplatelet count). The FCI accurately predicted fibrosis stages in HCV infected patients and seems more efficient than AAR, APRI, F1 and FIB-4 used serum indexes [53].

8.16 Forns index (Forns): Forns et al. (2002) developed an index derived from age, γ-glutamyl transferase (GGT), cholesterol, and platelet count in a study of 476 untreated HCV patients, which is calculated as follows: 7.811 - 3.131 × ln (platelet count(10^9/L)) + 0.781 × ln (GGT) + 3.467 × ln (age) - 0.014 × (cholesterol [mg/dL]). In their study, the AUROC for prediction of significant fibrosis (F2-F4 according to the Scheuer classification) was 0.86 in the test set and 0.81 in the validation set [54]. The diagnostic accuracy of this index has been confirmed in patients with HIV-HCV co-infection [55].

8.17 LOK (Model 3): Lok et al. (2005) proposed another prognostic model for prediction/exclusion of cirrhosis in patients with CHC [56]. This index can be derived from the regression formula: log odds = -5.56 - 0.0089 × platelet (× 10^9/L) + 1.26 × AST/ALT ratio + 5.27 × INR. Using model 3 at a cut-off of < 0.20, cirrhosis could be excluded with an NPV of 99%.

8.18 Fibrotest (Fibrosure): French investigators analyzed an extensive array of biochemical tests in 339 patients with CHC and identified a panel of 5 markers which could best predict the stage of fibrosis: α2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, and total bilirubin [57]. This test has been marketed as FibrotestTM in Europe and as FibrosureTM in the United States. The Fibrotest has been validated in several studies in patients with CHC and its diagnostic accuracy is limited by hemolysis (leading to a reduction in haptoglobin), Gilbert’s syndrome (increasing the bilirubin level), and recent or ongoing infection (leading to elevations of α2-macroglobulin and haptoglobin) [9].

8.19 FibroIndex: Japanese investigators proposed another index: 1.738 - 0.064 (platelets [× 10^9/mm^3]) + 0.005 (AST [IU/L]) + 0.463 (gamma globulin [g/dL]). The AUROC for prediction of significant fibrosis was 0.83 (0.82 in the validation set) which was slightly superior to APRI or the Forns index assessed in the same study [58].

8.20 Fibrometer: The Fibrometer test incorporates α2-macroglobulin, HA, AST, platelet count, prothrombin index, urea, and age. Cales et al. (2005) reported superior diagnostic accuracy of Fibrometer to Forns index, Fibrotest, and APRI [59]. However, this finding was not confirmed in an external validation study [60]. Several Fibrometers are now commercially available at BioLiveScale (Angers, France) for assessment of fibrosis in chronic viral hepatitis (Fibrometer V), alcoholic liver disease (Fibrometer A), and metabolic steatopathy (Fibrometer S).

8.21 Fibrospect II: The Fibrospect II assay (Prometheus Laboratories Inc., San Diego, CA) uses HA, TIMP-1 and α2-macroglobulin. This test was found to accurately predict significant fibrosis in a study of 294 HCV patients, validated in 402 HCV patients [61] and further validated in another study [59].

8.22 Enhanced liver fibrosis (ELF): In a study of 1021 patients with chronic liver disease of different etiologies, an algorithm consisting of age, HA, PIII-NP, and TIMP-1 was developed [62]. Using the Scheuer fibrosis score as a reference test, its overall diagnostic accuracy was similar to that of other non-invasive fibrosis tests (AUROC 0.78 for significant fibrosis, 0.89 for cirrhosis). Performance of the algorithm was slightly lower in a subgroup of patients with CHC (n = 325, AUROC 0.77 for prediction of F3-F4). This test is being marketed as Enhanced Liver Fibrosis (ELFTM) test by Siemens Medical Solutions Diagnostics (Tarrytown, NY).

8.23 Hepascore: It was developed by Australian investigators and is derived from bilirubin, GGT, HA, α2-macroglobulin, age and sex. High diagnostic...
performance was reported for both significant fibrosis and cirrhosis [63], but external validation yielded somewhat lower diagnostic accuracies [60].

8.24 TGF-β1, HA, PIIINP and TIMP-1 panel: Valva et al. (2011) studied the presence of a pro-fibrogenic cytokine (TGF-β1) as well as different matrix deposition markers [HA, PIIINP and TIMP-1] related to liver injury during CHC. They demonstrated that given the diagnostic accuracy of HA, PIIINP, TGF-β1, their combination may provide a potential useful tool to assess liver fibrosis in adults [64].

8.25 Fibroscan/Fibrotest: Castera et al. (2005) investigated APRI, Fibrotest, Fibroscan and combinations thereof in 183 patients with chronic hepatitis C. For significant fibrosis, the combination of Fibroscan and Fibrotest had superior diagnostic accuracy (AUROC 0.88) to that of respective individual tests [65].

8.26 Sequential algorithms: The sequential use of several markers may overcome some of the limitations of individual markers. Sebastiani et al. (2006) developed three different sequential algorithms (including APRI, Fibrotest and/or Forns index) for the diagnosis of significant fibrosis with elevated ALT, significant fibrosis with persistently normal ALT, and cirrhosis, respectively, which allowed classifying 100% of the patients for each entity [66].

8.27 Predicted liver fibrosis score (PLF score): It is derived from Fibroscan and multiple serological tests. It is calculated as: PLF score = 0.956 + 0.084 × TE - 0.004 × King score + 0.124 × Forns score + 0.202 × APRI score. A direct correlation was found to exist between PLF scoring system and the Metavir scoring system (P < 0.0001). The correlation of this scoring system with the severity of fibrosis was better than that of the other methods alone. The mean PLF scores for different stages of fibrosis ranged from 1.93 ± 0.45 for F0 to 3.64 ± 0.55 for F4. While there was no significant difference between mean PLF scores for F0 and F1 stages of fibrosis [67].

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<th>Calculation/Variables</th>
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<td>Wu index [69]</td>
<td>HA and INR[Wu index = (INR×HA/100)]</td>
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Plasma caspase-generated cytokeratin-18 fragments (CK-18) [76]

Fibroscan/Fibrotest [77] FibroTest™ (FT) and Transient Elastography (TE) (FibroScan®)

BMI, body mass index; HA, hyaluronic acid; INR, prothrombin time expressed as international normalized ratio; GGT, gamma glutamyl transferase; sFas, soluble Fas; TGF-β1, transforming growth factor β1; PIIINP, procollagen type III aminoterminal peptide.

9. Recent serum fibrosis markers (Table 3)

9.1 Serum bile acid (SBA) model: Halfon et al. (2013) analyzed SBA levels of 135 patients with CHC infection and correlated these levels with the degree of liver fibrosis determined by liver biopsy. They assessed the accuracy of SBA levels as predictor of liver fibrosis by comparison to patients’ Fibrotest scores. The SBA levels were significantly higher in patients with severe Fibrosis (Metavir F3-F4) compared to non-severe fibrosis (Metavir F0-F2). Furthermore, the ROC curve based on a new model that included serum bile acids, age, BMI, serum AST, glucose and cholesterol levels suggested that this combination reliably predicts the degree of liver fibrosis with high degree of accuracy, and is not inferior to the Fibrotest score [68].

9.2 Wu index based on prothrombin time as INR and HA: Wu et al. (2013) introduced a new simple index can easily predict significant liver fibrosis with a high degree of accuracy. It is calculated as Wu index = (INR×HA/100) to discriminate between F2-F4 patients and F0-F1 patients. It showed AUROC value of 0.921, and was better than APRI and FIB-4 [69].

9.3 AngioScore (AS): Hernández-Bartolome et al. (2013) developed a novel index based on serum levels of angiopoietin-2 measured in 108 CHC patients and validated in 71 CHC patients. AngioScore is calculated as follows: AS = -6.634 + (1.083xlog Ang2) + (1.792xlog Age) + (3.782xlog INR) + (1.052xlog AST) – (0.005xplatelets) + (0.653xlog GGT). The accuracy of this model was compared with APRI, FIB4, King, AAR, GUCI, Lok, Forns, Fl, and FCI. The model classified CHC patients by discarding significant fibrosis and diagnosing moderate and severe fibrosis (AUROC 0.886, 0.920, 0.923, respectively) with greater accuracy, sensitivity, and specificity. Therefore, it outperforms other indices and should help substantially in managing CHC and monitoring long-term follow-up prognosis [70].

9.4 Carbohydrate 19.9 antigen (CA19.9): CA19.9 is a glycoprotein expressed by several epithelial cancers, as well as in normal pancreatic and biliary duct epithelia, and it is used currently in the diagnosis and follow-up of gastrointestinal tumors. In a study of Bertino et al. (2013) on 180 patients, 116 with HCV-related chronic liver disease and 64 with HBV-related chronic liver disease, they suggested that elevation of CA 19.9 is related to the severity of fibrosis and to the viral etiology of hepatitis. They proposed that CA19.9 could be used in the combinations with the other markers already in use, in order to increase the diagnostic accuracy of the available tests, rising both PPV and NPV predictive values [71].

9.5 Combination of sFas with TGF-β1, HA, PIIINP: Valva et al. (2013) proposed the addition of apoptosis markers, particularly sFas combined with TGF-β1, HA, PIIINP in adult patients to more accurately assess liver fibrosis severity. The diagnostic accuracy evaluation demonstrated a good performance for sFas to evaluate advanced fibrosis in adults (AUROC: 0.8) [4].

9.6 Soluble CD163 (sCD163) and soluble mannose receptor (sMR, sCD206): Macrophages regulate the
fibrotic process in chronic liver disease. CD163 is an endocytic receptor for haptoglobin-hemoglobin complexes and is expressed solely on macrophages and monocytes. As a result of ectodomain shedding, the extracellular portion of CD163 circulates in blood as a soluble protein (sCD163) [72]. In a pilot study of Andersen et al. (2014), two new macrophage-specific serum biomarkers [sCD163 and sMR, sCD206] were evaluated as potential fibrosis markers in 40 CHC patients. The plasma concentrations of both biomarkers were significantly higher in infected patients and in cirrhosis compared to those with no/mild liver fibrosis (5.77 mg/l vs. 2.49 mg/l and 0.44 mg/l vs. 0.30 mg/l for sCD163 and sMR, respectively). The best separation between groups was obtained by sCD163 (AUC 0.89) as compared to sMR (AUC 0.75). sCD163 and sMR correlated significantly ($r (2) = 0.53$, $p<0.0001$). Interestingly, sCD163 was also correlated significantly with TNF-α. Thus, sCD163 is a promising new fibrosis marker in patients infected with HCV [73].

9.6 CD163-HCV-FS and CD163-HBV-FS fibrosis scores: Recently, Kazankov et al., investigated sCD163 in 551 patients with CHC and 203 patients with CHB before anti-viral treatment. sCD163 was associated with fibrosis stages for both HCV and HBV patients, with highest levels in patients with advanced fibrosis and cirrhosis. sCD163 was a marker of fibrosis independent of other biochemical parameters and known risk factors. They created two novel sCD163-based fibrosis scores, CD163-HCV-FS and CD163-HBV-FS, which showed AUROC of 0.79 and 0.71, respectively, for significant fibrosis. Compared to existing scores, CD163-HCV-FS was significantly superior to the APRI for all fibrosis stages and to FIB-4 for significant fibrosis, but CD163-HBV-FS was not [74].

9.7 Hyperglycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA*-M2BP): Serum WFA*-M2BP values were evaluated in 200 patients with chronic liver diseases. There were significant differences between fibrosis stages F1 and F2, and between F2 and F3. AUROC for the diagnosis of fibrosis ($F \geq 3$) using serum WFA*-M2BP value (0.812) was superior to the other surrogate markers, including APRI (0.694), HA (0.683), and type 4 collagen (0.625) [75].

9.8 Plasma caspase-generated cytokeratin-18 fragments (CK-18): Cusi et al. in a study on 424 patients, proposed CK-18 as a non-invasive alternative for liver biopsy in diagnosing and staging fibrosis. The CK-18 AUROC to predict fibrosis was 0.68 and the overall sensitivity/specificity was 85% [76].

9.9 Fibroscan/Fibrotest: FibroTest™ (FT) and Transient Elastography (TE) (FibroScan®) have been validated as non-invasive markers of METAVIR fibrosis stages from F0 to F4 using biopsy, and as prognostic markers of liver related mortality in patients with CHC. Poynard et al. extended the validation of FT and TE as markers of critical steps defined by occurrence of cirrhosis without complications (F4.1), esophageal varices (F4.2), and severe complications (F4.3): primary liver cancer, variceal bleeding, or decompensation (ascites, encephalopathy, or jaundice). At 5 years, among 501 patients without varices at baseline (F4.1) varices occurred in 19 patients [F4.2]. The predictive performance (AUROC) of FT was 0.77. At 10 years severe complications occurred in 203 patients, [F4.3], including primary liver cancer in 84 patients. FT was predictive of severe complications [AUROC 0.79], including primary liver cancer [AUROC 0.84]. Similarly TE was predictive of severe complications [AUROC 0.77], including primary liver cancer [AUROC 0.86] [77].

10. Limitations of fibrosis serum biomarkers
1. They have a tendency to be more elevated in the presence of inflammation; they are not liver-specific where they can detect fibrogenesis in organs other than the liver [17].
2. Serum marker readings may be falsely high due to their low clearance rates [17].
3. Some of these markers are not routinely available in most clinical laboratories [3].

Based on the above mentioned data in this review about different serum markers and their panels used for assessment of liver fibrosis we can become aware of some important facts. Firstly, the performance of simple tests derived from routine laboratory parameters appears to be similar to that of more complex and expensive panels. Almost all tests showed a better performance for diagnosing cirrhosis (AUROC 0.97-0.80) than for significant fibrosis (AUROC 0.76-0.89). Among non-invasive methods, based on serum markers, some gained a strong clinical foothold such as serum-based APRI, Fibrometer, Fibrotest and Fibroscan/Fibrotest. Some tests have limited clinical acceptance such as that based on serum PON-1 or PGAA. There are many other tests that are not yet widely validated such as TGF-β1, HA, PIIINP and TIMP-1 panel, SBA model, AngioSore, sCD163, and WFA+/M2BP, but remain promising. Most non-invasive tests fail to differentiate between early stages of fibrosis (F0 and F1-2) such as that based on serum CTGF, FibroQ, FIB-4, and PLF score. In fact, most of these tests can primarily distinguish cirrhosis from no or minimal fibrosis. Moreover, validity of some non-invasive tests (e.g. Fibrotest and Fibroscan) is extended recently to make them predictive markers for different cirrhosis complications too. Searching for tests able to differentiate accurately between F1 and F2 fibrosis stages is still needed.

CONCLUSION
The current utility of non-invasive diagnostics remains limited to pre-screening allowing physician to narrow the population of patient before definitive testing of liver fibrosis by biopsy of the liver. Thus, more studies on serum fibrosis markers and more validation efforts on large cohorts of patients with chronic liver diseases are needed. In addition, the use of a standardized system to evaluate the utility of serum markers would facilitate their introduction in clinical practice.

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