Review

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Serum biomarkers for liver fibrosis assessment

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Abstract: Liver fibrosis is the result of chronic liver injury of different etiologies produced by an imbalance between the synthesis and degeneration of the extracellular matrix and dysregulation of physiological mechanisms. Liver has a high regenerative capacity in the early stage of chronic diseases so a prompt liver fibrosis detection is important. Consequently, an easy and economic tool that could identify patients with liver fibrosis at the initial stages is needed. To achieve this, many non-invasive serum direct, such as hyaluronic acid or metalloproteases, and indirect biomarkers have been proposed to evaluate liver fibrosis. Also, there have been developed formulas that combine these biomarkers, some of them also introduce clinical and/or demographic parameters, like FIB-4, non-alcoholic fatty liver disease fibrosis score (NFS), enhance liver fibrosis (ELF) or Hepamet fibrosis score (HFS). In this manuscript we critically reviewed different serum biomarkers and formulas for their utility in the diagnosis and progression of liver fibrosis.

Keywords: liver fibrosis; liver fibrosis biomarkers; non-invasive biomarkers; serum biomarkers

Introduction

Liver fibrosis is the result of chronic liver injury of different etiologies, including viral hepatitis, alcohol abuse, metabolic diseases such as non-alcoholic fatty liver disease (NAFLD) now known as metabolic dysfunction-associated steatotic liver disease (MASLD) [1], autoimmune diseases, and cholestatic liver diseases [2, 3]. It is produced by dysregulation of physiological remodeling mechanism, activation of myofibroblasts, and formation of a fibrous scar that may eventually lead to the development of cirrhosis [4]. The common feature in liver fibrosis pathologies represents an imbalance between the synthesis and degeneration of the extracellular matrix (ECM) that affects its structure and properties [5]. The liver has a high regenerative capacity; however, when the damage occurs persistently, this regeneration develops into chronic diseases, such as fibrosis, which is characterized by excess accumulation of ECM [6, 7]. Liver fibrosis can be reversible especially in the early stage [4] before cirrhosis and organ failure, so it is important to diagnose it as soon as possible to establish an adequate treatment. Whereas in advanced liver disease there is impaired liver regeneration in both experimental models and patients [8].

Liver injury causes hepatocyte damage and disturbs tissue homeostasis, generally accompanied by inflammation [9]. When this situation occurs, it cause a pro-inflammatory response of Kupffer cells and an infiltration of immune cells that favor the activation of hepatic stellate cells (HSCs) into collagen-producing myofibroblasts [10, 11]. HSCs are the main controllers of ECM turnover, a process normally balanced by anti-fibrotic mechanisms that inactivate myofibroblast or stimulate its apoptosis [10]. In chronic liver diseases activated myofibroblast conduce to a downregulation of matrix metalloproteinases (MMPs), upregulation of MMP-inhibitors (TIMPs), and secretion of wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA-MZBP) [12], which are implied in ECM degradation. MMPs are the main enzymes implicated in ECM degradation and TIMPs are capable of regulating the proteolytic activities of MMPs in tissues [13]. In addition, activated-HSC are the most important contributors to collagen deposition in the space of Disse, which results in gradual thickening of the space causing an increase in the portal pressure. Thus, excessive collagen accumulation occurs and the matrix regeneration fails, leading to an increase in liver stiffness [14] (Figure 1).

Currently, the gold standard for assessing the degree of liver fibrosis remains liver biopsy, using histopathological...
scoring systems such as METAVIR, the most widely used, which establishes four stages of liver fibrosis progression as follows: F0, no fibrosis; F1, mild fibrosis (portal fibrosis without septa); F2, moderate fibrosis (portal fibrosis and few septa); F3, advanced fibrosis (numerous septa without cirrhosis); and F4, cirrhosis [15]. Liver biopsy has well-known limitations including invasiveness, poor acceptability, sampling variability, cost, and inter-observer variation in interpretation [16]. The development and use of other non-invasive biomarkers that can be used in the diagnosis and progression of liver disease is therefore necessary.

Here we critically review liver fibrosis blood biomarkers considering direct biomarkers those derived directly from the ECM formation and degradation process or the molecular pathogenesis of fibrogenesis and fibrinolysis, while indirect biomarkers include biochemical parameters that reflect alterations in liver function and liver injury [17].

**Indirect biomarkers**

**Liver enzymes**

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) provide information about hepatocyte injury. Decreased levels of ALT [18] while increased AST and AST/ALT ratio are found in advanced liver disease patients [19]. Also, up to 80 % of MASLD patients have aminotransferase concentrations within the normal range, so they are not considered reliable and accurate predictors for its diagnosis [20]. Furthermore, it has been shown that patients with normal ALT levels and alterations in glucose metabolism and insulin resistance could develop MASLD; likewise, normal aminotransferases results are not consistent criterion to exclude patients for further studies, such as image techniques or liver biopsy [21]. Also AST/ALT ratio has been used to establish cirrhosis risk in patients with chronic viral hepatitis [22, 23], non-alcoholic steatohepatitis (NASH), alcoholic liver disease (ALD) [24, 25] and primary biliary cirrhosis (PBC) [26], but nowadays, this ratio cannot be used alone in predicting different fibrosis stages as it does not discriminate between moderate fibrosis or severe fibrosis [27, 28].

**AST to platelets ratio index**

The AST to platelet ratio index (APRI), was initially evaluated to assess whether patients with HCV had liver fibrosis or not [25–29], and to differentiate between liver fibrosis stages and cirrhosis [30, 31]. A modified APRI (m-APRI) which incorporates age and serum albumin levels in the APRI formula has been proposed (Table 1) [32]. This m-APRI has been shown to improve the prediction of advanced fibrosis and cirrhosis in viral hepatitis [33].

**BARD**

This score was proposed by Harrison et al., taking into consideration the presence of type 2 diabetes mellitus, the patient’s body mass index (BMI) and liver serum enzymes
activity using the AST/ALT ratio [34]. BARD score has a high negative predictive value (NPV) of around 96% in MASLD patients [34]. Recently Park et al. [35] have reported in patients with MASLD the association between advanced liver fibrosis assessed by the BARD score, and an increased risk for cardiovascular disease (CVD) and mortality suggesting its relation with myocardial inflammation and ischemic stroke [35].

### Forns index

Forns index is a score system which combines age, gamma-glutamyltranspeptidase (GGT), cholesterol, and platelet count that has proved to be useful to identify patients without moderate hepatic fibrosis in chronic hepatitis C population [36]. Also, Forns index has been validated in biopsy proven MASLD patients in chronic liver disease population without decompensated cirrhosis or hepatocellular carcinoma [37]. Moreover, Romero et al. [38] described that in patients with genotype 1 CHC Forns index used in combination with APRI, shows a 95.2% accuracy in predicting moderate fibrosis and a 91.7% accuracy in detecting advanced fibrosis. Thus, Forns index has been shown as an accurate predictor of morbidities and mortality in MASLD patients, similar to APRI [39].

### FIB-4

FIB-4 is an index based on age, AST, ALT serum activities and platelet concentration (Table 1). It is probably the most widely used serum index for screening hepatic fibrosis as a first step, and its use is well recommended in MASLD clinical guidelines such as the American Association for the Study of Liver Diseases (AASLD) Practice Guidance on the Clinical Assessment and Management of Nonalcoholic Fatty Liver Disease [1, 40]. The most accepted FIB-4 cut-off for advanced fibrosis is 2.67 [41] but some studies have established it in 3.25 [42] (Table 2). Itakura et al. [43] found that FIB-4 has an accuracy rate of around 71% in the diagnosis of cirrhosis due to HBV infection, and around 75% in patients with HCV.

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**Table 1:** Scores and formulas used in liver fibrosis assessing and staging.

<table>
<thead>
<tr>
<th>Score/index</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRI</td>
<td>AST (IU/L)/upper limit of normal AST value (IU/L)/platelet count (10^9/L) × 100</td>
</tr>
<tr>
<td>m-APRI</td>
<td>Age (years) × (AST (IU/L)/upper limit of normal AST value (IU/L))/platelet count (10^9/L) × 100</td>
</tr>
<tr>
<td>BARD score</td>
<td>AST/ALT &gt; 0.8 2 points</td>
</tr>
<tr>
<td>Forns index</td>
<td>AST/ALT &gt; 0.8 2 points</td>
</tr>
<tr>
<td>NFS</td>
<td>−1.675 + (0.037 × age (year)) + (0.094 × BMI (kg/m^2)) + (1.13 × IFG/diabetes (yes = 1, no = 0)) + (0.99 × AST/ALT ratio) − (0.013 × platelets (10^9/L)) − (0.66 × albumin (g/dL))</td>
</tr>
<tr>
<td>FibroTest</td>
<td>(4.467 × log (a2-MG)) − (1.357 × log (haptoglobin)) + (1.017 × log (GGT)) − (0.0281 × age (year)) + (1.737 × log (total bilirubin)) − (1.184 × apoA1) + (0.301 × sex (male = 1, female = 0)) − 5.540</td>
</tr>
<tr>
<td>Fibrometer NAFLD</td>
<td>(0.4184 × glucose (mmol/L)) + (0.0701 × AST (IU/L)) + (0.0008 × ferritin (μg/L)) − (0.0102 × platelet (10^9/L)) − (0.0260 × ALT (IU/L)) + (0.0459 × body weight (kg)) + 0.0842 × age (year)) + 11.6226</td>
</tr>
<tr>
<td>Hepascore</td>
<td>Where Y = exp (−4.185818 − (0.0249 × age) + (0.7464 × sex) + (1.0039 × a2-MG) + (0.0302 × HA) + (0.0691 × total bilirubin) − (0.0012 × GGT))</td>
</tr>
</tbody>
</table>
| HFS         | 1/1 + e^−[(3.390 − 0.986 × age [45–64 years of age] = 1.719 × age [≥65 years of age] + 0.875 × male sex − 0.896 × AST [35–69 IU/L] − 2.126 × AST [≥70 IU/L] − 0.027 × albumin [4.4–9.4 g/dL] − 0.897 × albumin [≤4.4 g/dL] − 0.899 × HOMA–R [2 − 3.99 with no diabetes mellitus] − 1.497 × HOMA–R [≥4 with no diabetes mellitus] − 2.184 × diabetes mellitus − 0.882 × platelets × 1.000μL [155–219] − 2.233 × platelets × 1.000μL [≥255])]
| Benlloch index | 1/1 + e^−12.668 + (0.017 × (albumin/total protein ratio) − (1.356 × (prothrombin time)) − (0.004 × (AST)) − (0.02 × (time since liver transplantation)) |
| ADAPT score | 

$$
\text{exp} (\log_{10} (\text{age} \times \text{PRO-C3/platelet count}) + \text{diabetes} (0 = \text{absent}; 1 = \text{present}))
$$

| ELF         | 2.494 + 0.846 ln (HA) + 0.735 ln (PIIINP) + 0.391 ln (TIMP-1) |
| CHE3L1 model | 0.032 × AST − 0.012 × platelets + 0.012 × HA + 0.064 × log (CHE3L1) − 4.752 |
| M2BPGi COI  | ([M2BPGi]_sample − [M2BPGi]_negative control)/([M2BPGi]_positive control − [M2BPGi]_negative control) |

α2-MG, alpha 2 macroglobulin; ALT, alanine aminotransferase; ApoA1, Apolipoprotein A1; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CHE3L1, Chitinase 3-like protein 1; ELF, enhanced liver fibrosis; GGT, gamma-glutamyltranspeptidase; HA, hyaluronic acid; HFS, heparmot fibrosis score; HOMA-R, homeostatic model assessment of insulin resistance; m-APRI, modified APRI; M2BPGi, Mac-2 binding protein glycosylation isomer; M2BPGi, Mac-2 binding protein glycosylation isomer cut-off index; NFS, non alcoholic fatty liver disease fibrosis score; IFG, impaired fasting glucose; PIIINP, propeptide of type III procollagen; TIMP-1, matrix metalloproteinases inhibitor type 1.
infection. Although different meta-analyses also found that FIB-4 as well as APRI were moderately effective for the assessment of fibrosis stage in chronic hepatitis B [44–46], FIB-4 has a higher diagnostic accuracy when compared with APRI for predicting moderate or advanced fibrosis and cirrhosis diagnosis [46]. As reported in different studies, FIB-4 has a high diagnostic value to assess cirrhosis, and moderate or severe fibrosis [47–54]. FIB-4 is a useful tool in liver fibrosis screening because of its practicability and high NPV [55]. Thus, a cut-off of 1.3 has been proposed to discard advanced liver fibrosis [56]. However, the specificity for advanced fibrosis in patients aged ≥65 years is lower, resulting in a high false positive rate, so the proposed FIB-4 cut-off in this group of age increase to 2 [57]. Although the usefulness of this biomarker to rule out advanced liver fibrosis, in patients with a high prevalence such as diabetics, MASLD cannot be ruled out with a single FIB-4, if there is high suspicion, re-evaluation, or use of other more specific methods, is recommended [58, 59].

**NAFLD fibrosis score**

NAFLD fibrosis score (NFS) considers the same parameters as FIB-4 plus BMI, diabetes (yes/no) and albumin (Table 1). Its use has been recommended by the EASL-EASD-EASO Clinical Practice Guidelines [81] for MASLD patients to whether diagnose advanced liver fibrosis or not, as it has an area under the receiving operating characteristics curve (AUROC) >0.8, similar to FIB-4 (Table 2) [60, 63]. However, despite their practicability FIB-4 and NFS are still considered suboptimal as they have a substantial proportion of false-positive and false negatives when applied to the general population, thereby they should only be used in at-risk populations [82].

**Fibrotest**

FibroTest™ (Biopredictive Paris), is a multimarker panel consisting of serum α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and GGT, adjusted for age and gender [83] (Table 1). Fibrotest has been validated in common liver diseases such as chronic hepatitis B (CHB) [84], Alcoholic liver disease (ALD) [85], and MASLD [83] for stratifying liver fibrosis. In a recent meta-analysis Vali et al. [86] concluded that Fibrotest has acceptable performance in detecting cirrhosis (AUC = 0.92) in MASLD patients, however, it showed limited accuracy in predicting moderate and advanced fibrosis (AUROC = 0.77 for both conditions). Similar results were found in chronic viral hepatitis patients by Degos et al. [61] (Table 2). Despite all, Fibrotest has good predictive values for diagnosing liver fibrosis in MASLD patients and thus it is included in EASL-EASD-EASO Clinical Practice Guidelines [81], also for the survival without liver-related deaths, the CVD-related deaths and the overall survival [87].

**Golgi protein 73 (Gp73)**

Gp73 is a transmembrane protein released by damaged cells, increased in the serum of chronic liver patients [88–90]. It is known to be highly expressed in patients with liver cirrhosis [90], and thus, it has shown great diagnostic value for liver cirrhosis [91, 92]. Recently, it has been demonstrated that in patients with compensated cirrhosis higher levels of Gp73 are related to worse outcomes such as decompensation, hepatocarcinoma development, and liver-related deaths [93, 94]. Its use also has been validated in MASLD [95], ALD and viral hepatitis patients [64, 88, 96], proving to be a useful tool in the diagnosis of advanced fibrosis and cirrhosis, even better than FIB-4 or APRI [97].

**Hepamet fibrosis score (HFS)**

HFS is a recently proposed new score validated in a large, multicenter European population of Caucasian ethnicity with biopsy-proven MASLD that includes age, sex, diabetes, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), AST, albumin, and platelets in its formula [65]. Its developers have reported that HFS identified patients with advanced fibrosis with greater accuracy compared to FIB-4 and NFS index. Although it is a novel score, several studies had validated its thresholds [98–100] and verified that HFS had the highest diagnostic accuracy and the highest negative predictive value when compared to NFS and FIB-4 in metabolic hepatic steatosis patients [101]. Moreover, HFS is as reliable as NFS, and FIB-4 for predicting cirrhosis, long-term liver-related events, hepatocarcinoma, and overall mortality, with higher performance in the prediction of moderate and severe fibrosis, explained by the fact of including the presence of diabetes in its formula or in non-diabetics patients, the HOMA-IR [102]. Moreover, higher levels of HFS (cut-off 0.12) are related to an increased risk of developing type 2 diabetes mellitus and arterial hypertension in MASLD patients, but NFS or FIB-4 could not predict this outcome [103].

**OWLiver**

The patented metabolomic test OWLiver® (One Way Liver S.L., Bilbao, Spain) is a fasting blood test able to measure the
<table>
<thead>
<tr>
<th>Score/index</th>
<th>Patients/cohort</th>
<th>Diagnosis</th>
<th>Predict/rule out</th>
<th>AUC</th>
<th>Cut-off</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
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<tbody>
<tr>
<td>AST/ALT</td>
<td>MASLD</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.83</td>
<td>&gt;0.8</td>
<td>74</td>
<td>78</td>
<td>44</td>
<td>93</td>
<td>[60]</td>
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<td>APRI</td>
<td>Patients with chronic viral hepatitis</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.72</td>
<td>&gt;0.5</td>
<td>79.9</td>
<td>48.4</td>
<td>67.3</td>
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<td>Predict</td>
<td>0.67</td>
<td>&gt;1</td>
<td>27</td>
<td>89</td>
<td>37</td>
<td>84</td>
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<td>MAPRI</td>
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<td>79.8</td>
<td>75.7</td>
<td>81.6</td>
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<td>44</td>
<td>27</td>
<td>95</td>
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<td>FORNS INDEX</td>
<td>Advanced fibrosis</td>
<td>Rule out</td>
<td>0.86</td>
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<td>85</td>
<td>65</td>
<td>36</td>
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<td>[42, 60]</td>
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<td>&gt;3.25</td>
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<td>85</td>
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<td>0.737</td>
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<td>71.2</td>
<td>38</td>
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<td>43</td>
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<td>Rule out</td>
<td>Predict</td>
<td>&gt;2.67</td>
<td>33</td>
<td>98</td>
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<td>&gt;0.676</td>
<td>33</td>
<td>98</td>
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<td>Patients with chronic viral hepatitis</td>
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<td>Predict</td>
<td>0.82</td>
<td>&gt;0.74</td>
<td>62.6</td>
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<td>Gp73</td>
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<td>0.806</td>
<td>&gt;85.7 ng/mL</td>
<td>43.59</td>
<td>97.18</td>
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<td>0.742</td>
<td>&gt;84.49 ng/mL</td>
<td>30.70</td>
<td>96.23</td>
<td>89.74</td>
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<td>NR</td>
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<td>≤0.2</td>
<td>87</td>
<td>71</td>
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<td>HA</td>
<td>Chronic liver diseases</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;90 µg/L</td>
<td>80.4</td>
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<td>Chronic liver diseases</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;210 µg/L</td>
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<td>60.8</td>
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<td>PRO-c3</td>
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<td>95</td>
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<td>Predict</td>
<td>0.72</td>
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<td>71.9</td>
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<td>NR</td>
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<td>ADAPT score</td>
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<td>44</td>
<td>97</td>
<td>[62]</td>
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<td>&gt;6.328</td>
<td>NR</td>
<td>NR</td>
<td>48.4</td>
<td>96.6</td>
<td>[69]</td>
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### Table 2: (continued)

<table>
<thead>
<tr>
<th>Score/index</th>
<th>Patients/cohort</th>
<th>Diagnosis</th>
<th>Predict/rule out</th>
<th>AUC</th>
<th>Cut-off</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
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<td>CH3L1 OR YKL-40</td>
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<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.764</td>
<td>&gt;165 µg/L</td>
<td>70</td>
<td>76.8</td>
<td>NR</td>
<td>NR</td>
<td>[70]</td>
</tr>
<tr>
<td>ALD</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;330 µg/L</td>
<td>88.5</td>
<td>50.8</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>[71]</td>
</tr>
<tr>
<td>HBV</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.97</td>
<td>&gt;68.75 µg/L</td>
<td>95.2</td>
<td>89.7</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>[72]</td>
</tr>
<tr>
<td>HCV</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.809</td>
<td>&gt;186.4 µg/L</td>
<td>78</td>
<td>81</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>[73]</td>
</tr>
<tr>
<td>M2BPGi COI</td>
<td>HBV</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.653 (0.608-0.698)</td>
<td>&gt;0.25</td>
<td>74.8</td>
<td>47.3</td>
<td>NR</td>
<td>NR</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>HBV</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.59 (0.50-0.67)</td>
<td>≥3.0</td>
<td>18.8</td>
<td>98.5</td>
<td>NR</td>
<td>NR</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Predict</td>
<td>0.795 (0.743-0.848)</td>
<td>&gt;0.45</td>
<td>69.6</td>
<td>74.1</td>
<td>NR</td>
<td>NR</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>HBV</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>0.914 (0.815-1)</td>
<td>&gt;0.96</td>
<td>83.3</td>
<td>92.7</td>
<td>NR</td>
<td>NR</td>
<td>[74]</td>
</tr>
<tr>
<td>ELF</td>
<td>Chronic liver diseases</td>
<td>Severe fibrosis</td>
<td>Predict</td>
<td>0.86 (0.83-0.89)</td>
<td>≥10.48</td>
<td>62</td>
<td>89</td>
<td>73</td>
<td>83</td>
<td>[76]</td>
</tr>
<tr>
<td>Liver fibrosis (EUROGOLF cohort)</td>
<td>Mild fibrosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;7.7</td>
<td>85</td>
<td>38</td>
<td>NR</td>
<td>NR</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>Liver fibrosis (EUROGOLF cohort)</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;9.8</td>
<td>65</td>
<td>90</td>
<td>NR</td>
<td>NR</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>Liver fibrosis (EUROGOLF cohort)</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>NR</td>
<td>≥11.3</td>
<td>38</td>
<td>97</td>
<td>NR</td>
<td>NR</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>NR</td>
<td>&gt;9.8</td>
<td>62</td>
<td>66</td>
<td>55</td>
<td>72</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.81</td>
<td>≥0.55</td>
<td>82</td>
<td>65</td>
<td>70</td>
<td>78</td>
<td>[79]</td>
<td></td>
</tr>
<tr>
<td>Patients with chronic viral hepatitis</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.78 (0.75-0.80)</td>
<td>&gt;0.5</td>
<td>52.9</td>
<td>86.3</td>
<td>83.7</td>
<td>57.9</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.852 (0.778-0.926)</td>
<td>&gt;0.5</td>
<td>67</td>
<td>92</td>
<td>NR</td>
<td>NR</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>Advanced fibrosis</td>
<td>Predict</td>
<td>0.957 (0.918-0.995)</td>
<td>&gt;0.5</td>
<td>95</td>
<td>81</td>
<td>NR</td>
<td>NR</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>0.938 (0.872-1.000)</td>
<td>&gt;0.84</td>
<td>71</td>
<td>89</td>
<td>NR</td>
<td>NR</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>Patients with chronic viral hepatitis</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>0.86 (0.83-0.88)</td>
<td>&gt;0.84</td>
<td>59</td>
<td>87.4</td>
<td>43.2</td>
<td>92.9</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>Fibrometers</td>
<td>Patients with chronic viral hepatitis</td>
<td>Significant fibrosis</td>
<td>Predict</td>
<td>0.79 (0.76-0.81)</td>
<td>&gt;0.411</td>
<td>83.1</td>
<td>57.1</td>
<td>72</td>
<td>71.8</td>
<td>[61]</td>
</tr>
<tr>
<td>Fibrometers</td>
<td>Patients with chronic viral hepatitis</td>
<td>Cirrhosis</td>
<td>Predict</td>
<td>0.86 (0.83-0.89)</td>
<td>&gt;0.442</td>
<td>43.9</td>
<td>95</td>
<td>58.1</td>
<td>91.5</td>
<td>[61]</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; AUC, area under the curve; CH3L1, Chitinase 3-like protein 1; ELF, enhanced liver fibrosis; Gp73, Golgi protein 73; HA, hyaluronic acid; HFS, hepatomet fibrosis score; HBV, hepatitis B virus; HCV, hepatitis C virus; M2BPGi COI, Mac-2 binding protein glycosylation isomer cut-off index; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; NPV, negative predictive value; NR, not reported; OELF, original ELF; PIIINP, N-terminal propeptide of procollagen type III; PPV, positive predictive value.
degree of MASLD development by measuring a panel of triacylglycerols serum biomarkers and BMI, which represents the fat and inflammation of the liver [104]. The triacylglycerols are measured by high-performance liquid chromatography and mass spectrometry (UHPLC-MS) and then all the results are taken together in an algorithm that gives the final OWLiver® score [105]. Owliver is able to distinguish between normal liver and MASLD liver fibrosis showing an AUROC of 0.90 with a high sensitivity, which means a low rate of false negatives cases [106]. This score can also differentiate between simple steatosis and steatohepatitis pathology as reported in the prospective validation study, where patients had previously been diagnosed by liver biopsy [104]. When compared with liver biopsy, the OWLiver® Care and OWLiver® tests had a suboptimal performance in patients with type 2 diabetes mellitus patients, as reported by Bril et al. [107]. A lack of comparative studies between OWLiver® and other non-invasive liver fibrosis scores, and the complexity of its methodology makes it difficult to be implemented in clinical practice.

**Benlloch index**

Benlloch index is a model created to evaluate chronic HCV patients who have undertaken a liver transplantation. The aim of Benlloch index is to evaluate whether or not is necessary to start antiviral therapy and follow up carefully this group of patients in order to indicate retransplantation [66]. This index uses 4 indirect biomarkers AST, prothrombine time, albumin/total protein ratio, and time since liver transplantation (Table 1). It has demonstrated efficacy compared to liver biopsy in chronic HCV patients who have undertaken a liver transplantation showing an acceptable discriminative power differentiating significant and advanced fibrosis [66].

**Direct biomarkers**

**Extracellular matrix-derived proteins**

**Hyaluronic acid**

Hyaluronic acid (HA) is an important constituent of the extracellular matrix and is highly present in the liver [108]. Several cell types can secrete HA, the synovial lining cells and the HSC are responsible of its synthesis in the liver, while sinusoidal endothelial cells are involved in its degradation [109]. HA serum concentrations are elevated in liver diseases associated with fibrosis such as ALD [109, 110], MASLD [111–113], HCV [114–116], HBV [108, 117] and HIV-HCV coinfection [118, 119]. Therefore, it can be used as a non-invasive biomarker to assess the presence of liver fibrosis and to monitor disease progression [109, 120].

**N-terminal propeptide of procollagen type III**

N-terminal propeptide of procollagen type III (PCIIINP) is one of the major components of connective tissue that has been recently reported to perform well in the detection of fibrosis in type 2 diabetes patients [121]. Serum levels PCIIINP are increased in ALD or HCV patients and it correlates with the stage of liver fibrosis [122–124]. In addition, PCIIINP plasma levels have been studied and showed to perform better than APRI or FIB-4 as a non-invasive biomarker for diagnosing liver fibrosis stage in children and adolescents with biopsy-proven MASLD [125].

**Laminin and type IV collagen**

Type IV collagen (CIV) and laminin (LN), have been widely evaluated in ALD, viral hepatitis, and MASLD patients [111, 126]. In addition, serum CIV and LN levels correlated with the fibrotic stage in HCV patients and were reported as accurate non-invasive markers of liver fibrosis and liver inflammation [127]. Otherwise, LN has a lower diagnostic performance compared to HA and CIV [128].

Serum HA, PCIIINP and CIV have been proposed as biomarkers for an accurate diagnosis of liver fibrosis in different chronic liver diseases, furthermore, HA is the best for screening liver cirrhosis [67]. However, Stefano et al. reported that CIV could predict the presence of moderate and advanced fibrosis in MASLD patients with a better AUROC than LN, HA, and PCIIINP [129].

**N-protease cleavage site of PIIINP**

N-protease cleavage site of PIIINP (PRO-C3) is a systemic marker of type III collagen formation and fibroblast activity. Thus, is therefore directly related to the development of liver fibrosis. It has been proved to detect liver fibrosis, progression rate and treatment response in patients with chronic liver disease [68, 130–132]. PRO-C3 was firstly proved to differentiate moderate from severe fibrosis in CHC patients and, also identify CHC patients with fibrosis progression more accurately than the common used FibroTest [68]. Moreover, it has been found that in ALD and MASLD population the utility of PRO-C3 increases when used in an algorithm known as the ADAPT score, which includes age, diabetes and platelets count in its formula (Table 1) [62, 69]. The ADAPT score shows superiority when compared to APRI,
FIB-4 and NFS, and also has the advantage of being able to stratify fibrosis or cirrhosis in contrast to the other non-invasive biomarkers, which only allow dichotomous results [69].

Matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs)

TIMP-1 was the only metalloproteinase that could be considered an independent predictor of histological fibrosis as reported in a study conducted on MASLD patients [133]. However, Livzan et al. reported TIMP-2 as a potential non-invasive marker for the diagnosis of liver fibrosis in patients with MASLD having a good correlation with the severity of fibrosis [134]. Also, Boeker et al., reported that MMP-2 can be used to detect cirrhosis with high efficacy in patients with chronic HCV, and that performed better than HA or TIMP-1 [135]. Furthermore, the MMP-2/TIMP-1 ratio was proposed as an indicator of Interferon-γ treatment response in CHC patients, describing a greater decrease in ratio levels in responders compared to no-responders or not treated patients [136]. In addition, Munsterman et al. described higher levels of TIMP-1 and TIMP-2 in severe fibrosis than in mild or no fibrosis in MASLD patients, and also that MMP-9 was the only ECM component correlated with inflammation severity [137]. Moreover, MMP-7 has been recently described as an independently associated liver fibrosis biomarker capable of improving the diagnostic performance in older MASLD patients when combined with the enhanced liver fibrosis (ELF) test [138].

Chitinase 3-like protein 1 (CHI3L1)

CHI3L1 also called YKL-40, is a protein secreted by macrophages, neutrophils, vascular smooth muscle cells, cancer cells, etc. but its expression in the liver is higher than in other tissues. CHI3L1 has several functions as promoting extracellular matrix degradation and tissue remodeling [139]. CHI3L1 levels have been related to the staging of liver fibrosis in MASLD [70], ALD [71], HBV [72] and HCV patients [73]. Huang et al. showed that CHI3L1 is a good marker in differentiating substantial fibrosis (AUC = 0.94), and advanced fibrosis (AUC = 0.96). Also, that CHI3L1 is superior to HA, PIIINP, LN, and CIV for this purpose [140]. As reported by Saitou et al. [73], serum CHI3L1 levels outperform other non-invasive fibrosis biomarkers including CIV, HA, and PIIINP for distinguishing advanced fibrosis from mild fibrosis with an AUC of 0.809 in HCV infection patients and that its levels decrease after therapy. Furthermore, a CHI3L1 model has been proposed and it was found to be superior to APRI and FIB-4 in predicting moderate fibrosis in HBV patients with ALT less than two times the upper limit of the normal range [141].

Mac-2 binding protein glycosylation isomer (M2BPGi)

M2BPGi is a glycoprotein produced by HSC, which functions as a messenger between HSC and Kupffer cells promoting fibrogenesis [142]. Thus, it has been recommended as an accurate biomarker for staging hepatic fibrosis [143, 144]. M2BPGi levels are expressed as a cut-off index (COI) in literature, and its usefulness in liver fibrosis has been validated in several studies in patients with different aetiologies such as HVC [145], HBV [75, 146] (Table 2), autoimmune hepatitis [147], NASH [148], MASLD [111, 149], biliary atresia [150], primary biliary cirrhosis [151], primary sclerosing cholangitis [152] and mortality in liver cirrhosis [153]. In a study in HBV patients, M2BPGi correlated with the fibrosis stage (F0–F4) and was superior to PLT count, HA, PIIINP, TIMP-1, FIB-4 index, APRI, and ELF score for staging moderate fibrosis [154]. Similar results were found when compared to AST/ALT ratio, APRI and FIB-4 for detecting advanced liver fibrosis [74]. Furthermore, recent studies described M2BPGi as a biomarker in the follow-up after antiviral therapy in liver fibrosis patients [155–159], patients with high M2BPGi levels after antiviral treatment, must be followed up carefully for hepatocarcinoma development [155, 160]. Moreover, as reported in a study of HCV patients, M2BPGi was better than FIB-4 to distinguish different fibrosis stages after treatment with direct-acting antivirals [161].

Although the utility demonstrated of these direct biomarkers, they show better sensitivity and specificity when used combined [124], that is in algorithms or scores such as ELF, Hepascore or Fibrometer which will be described below.

Calculated formulas or index using extracellular matrix deriving proteins

Enhance liver fibrosis (ELF)

ELF is a patented blood test (Siemens Healthineers, Erlagen, Germany) that measures three molecules involved in liver matrix metabolism (TIMP-1, PIIINP and HA) to give a score reflecting the severity of liver fibrosis. Since its appearance as the original ELF (OELF), the algorithm has suffered some modifications such as eliminating the parameter age, thus different thresholds have been reported [162, 163]. ELF has revealed good accuracy in predicting liver fibrosis [77, 164], having been validated in different chronic liver diseases such as ALD [165], MASLD [166], primary biliary cirrhosis [167] and viral hepatitis infection [78, 168, 169]. It is capable of
distinguishing severe fibrosis, moderate fibrosis, and no fibrosis, with an AUC of 0.90, 0.82, and 0.76 respectively [170] (Table 2). Moreover, ELF as APRI and FIB-4 have been described to be useful in early identification of patients at high risk of severe post liver transplant hepatitis C recurrence [171]. Higher ELF levels have been related to worse clinical outcomes in patients with chronic liver disease, suggesting that could be used in prognostic [76]. The AASLD Practice guidance on the clinical assessment and management of MASLD, recommends its use as a second line specific test, being comparable to FibroScan in advanced liver fibrosis assessment [1, 172]. However, influence factors such as gender, age and time in which the blood test is conducted need to be taken into account when interpreting results to minimize the variability of the test [173].

**Hepascore**

Hepascore was first validated in predicting different stages of fibrosis among HCV infected patients. It combines socio-demographic variables like age and gender with blood-based parameters including bilirubin, gamma-glutamyl trans- ferase, HA, and α2-macroglobulin [80] (Table 1). Nowadays is a widely used algorithm to detect moderate fibrosis in many chronic liver diseases [79, 174, 175]. As reported by Huang et al. [174], Hepascore had a better diagnostic ability for moderate and advanced fibrosis in CHC, CHB and ALD than MASLD and HIV co-infected viral hepatitis. Furthermore, it has been compared to other scores, and it showed significantly higher diagnostic values in ALD patients than APRI, and FIB-4 scores, however, it was similar compared to FibroTest, FibrometerA [85]. Contrarily, Chrostek et al. [176], revealed that Hepascore had a lower diagnostic value in a randomized group of alcoholic patients than APRI, Forns and FIB-4 when using Fibrotest as a matrix for comparing their diagnostic values. On the other hand, in patients with MASLD, is capable to identify advanced liver fibrosis [177]. In addition, Hepascore has been shown to predict accurately long-term risks such as decompensation, and hepatocarcinoma, both liver-related death in patients with metabolic dysfunction associated with MASLD [178]. However, large biological within-individual variations in non-fasting plasma HA are found in both health and chronic liver disease, thus the Hepascore system should be evaluated with caution in single measurement or clinical follow-up [179].

**FibroMeters**

FibroMeters is a family of patented blood biomarkers panels with several specificities, flexible to be adapted according to the cause of the chronic liver disease [180]. They included direct blood biomarkers such as HA, α2-macroglobulin, and indirect blood test prothrombin time, platelets, AST, ALT, GGT, bilirubin, urea, ferritin and other data such as age, body weight and gender (Table 1). FibroMeters were first developed and validated for the detection of fibrosis stage in patients with CHB or chronic hepatitis C (CHC) [181] and MASLD [182]. FibroMeter test for fibrosis staging in CHC showed an AUROC significantly higher than Fibrotest, Hepascore, APRI and FIB-4, as reported by Càles et al. [180]. In a meta-analysis in CHC patients, FibroMeter demonstrated superiority over both FibroTest and Hepascore in terms of overall diagnostic performance [183]. The standard FibroMeter was expanded to improve the diagnostic performance for cirrhosis, which uses specific coefficients with the same clinical and blood parameters as the standard FibroMeter, resulting in a PPV of 100 % in HCV patients [184]. Moreover, in MASLD patients FibroMeter has shown higher accuracy for moderate fibrosis than APRI or NFS [185]. FibroMeter virus second (V2G) and third generation (V3G) are both two tests that were initially developed for the diagnosis of moderate fibrosis in patients with hepatitis C [186], but in recent years they have also been validated in patients with MASLD [182, 187]. The latest version is the FibroMeter vibration-controlled transient elastography (VCTE), which is a combination of FibroMeter V3G and TE [188]. It has shown the best diagnostic accuracy in detecting advanced fibrosis when compared to Fibrometer VG2 and Fibrometer MASLD [189] and also is better than NFS, and TE for diagnosing severe liver fibrosis in MASLD patients [190]. Guillaume et al. [187], reported equal accuracy for ELF and FibrometerV2G in patients with MASLD. In addition, FibroMeter VCTE had good diagnostic accuracy, similar to TE, for predicting severe fibrosis in autoimmune hepatitis (AIH) and performed even better in primary biliary cholangitis (PBC) as reported by Zachou et al. [191].

**Conclusions**

Establishing an early diagnosis of liver fibrosis at early stages is essential to perform an accurate clinical intervention and prevent the progression from liver fibrosis to liver cirrhosis and hepatocellular carcinoma. Also, it is important to diagnose it as soon as possible as liver fibrosis is associated with long-term overall mortality, liver transplantation, and liver-related events [192]. Serum biomarkers are a very good option since they allow continuous monitoring, and they are less invasive compared to liver biopsy. All these non-invasive scoring systems, including direct and indirect serum biomarkers, yield high sensitivity but poor specificity,
suggested that they are best applied to exclude subjects without advanced fibrosis in MASLD populations [81], thereby avoiding unnecessary liver biopsies but not being useful to establish an accurate diagnosis by themselves. The use of these serum biomarkers and indirect indexes in a first step, or a combination of serum biomarkers with specific index such as ELF or combined with FibroScan or TE, are reliable strategies well recommended in clinical guidelines to better perform the liver fibrosis staging in different etiologies patients [16], as is a cost-effective diagnosis process. Furthermore, some of these biomarkers such as FIB-4 or NFS are considered an easy and economic tool that if implemented could cause a high impact in catching patients with liver fibrosis at the initial stages, where this pathology could be reverted [193, 194]. Although all this non invasive biomarkers and indexes have been study in at-risk population, they could be use as screening in groups of patients in primary care, such as type 2 diabetes mellitus, alcohol users disorders, metabolic risk factors or elevated liver enzymes, as there is still an extensive prevalence of chronic liver diseases in the general population [195]. For this reason, the American Diabetes Association (ADA) and the European Associations for the Study of Diabetes (EASD), of the Liver (EASL) and of Obesity (EASO) recommend screening for advanced liver fibrosis in all type 2 diabetes mellitus patients [81, 196]. Thus, every laboratory should evaluate and define the best diagnosis strategy in consensus with clinicians to rend the highest diagnostic performance in their target population.

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fatty liver disease, compared to chronic hepatitis C, B, and alcoholic liver disease. Aliment Pharmacol Ther 2018;48:1117–27.


