Review Article

Noninvasive Biomarkers of Liver Fibrosis: An Overview

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Chronic liver diseases of differing etiologies are among the leading causes of mortality and morbidity worldwide. Establishing accurate staging of liver disease is very important for enabling both therapeutic decisions and prognostic evaluations. A liver biopsy is considered the gold standard for assessing the stage of hepatic fibrosis, but it has many limitations. During the last decade, several noninvasive markers for assessing the stage of hepatic fibrosis have been developed. Some have been well validated and are comparable to liver biopsy. This paper will focus on the various noninvasive biochemical markers used to stage liver fibrosis.

1. Introduction

Chronic liver diseases of differing etiologies are among the leading causes of morbidity and mortality worldwide [1–5]. Chronic liver disease progresses through different pathological stages that vary from mild hepatic inflammation without fibrosis to advanced hepatic fibrosis and cirrhosis [6–8]. Assessment of the stage of liver disease is important for diagnosis, treatment, and follow-up both during treatment and after cessation of treatment. A liver biopsy is the oldest and most accurate method used to evaluate liver histology and the progression of chronic liver disease. Furthermore, different histological scoring systems have been developed and modified [9–12]. A liver biopsy is considered the gold standard for assessing liver histology [7, 12–14]. During the pathological progression of liver fibrosis, excessive amounts of extracellular matrix build up; furthermore, serum levels of various biomarkers change, in addition to the appearance of new biomarkers in the serum during the different stages of fibrosis [7, 8, 15]. Recently many noninvasive markers (NIMs) for assessing liver fibrosis have been developed, and they are frequently used in clinical practice. They have been validated in different studies, and some were found to be highly accurate in the assessment of liver fibrosis compared with liver biopsies [16–19], which have always been used as the standard reference method for evaluating the accuracy of noninvasive methods.

2. Is the Liver Biopsy Really the Gold Standard and Reference Method for Evaluating Hepatic Fibrosis?

2.1. The Following Are Limitations of the Liver Biopsy. (1) The liver biopsy does not efficiently reflect the fibrotic changes occurring in the entire liver because an optimally sized biopsy contains 5–11 complete portal tracts and reflects only 1/50000 the volume of the liver. (2) The process of hepatic fibrosis is not linear, and biopsies from different areas have shown different stages of fibrosis. (3) Several reports have shown that cirrhosis may be missed in 10–30% of patients. (4) A liver biopsy cannot differentiate between early and advanced end-stage cirrhosis; thus, it cannot be used as an ideal prognostic predictor. (5) Disagreements between pathologists occur, which may correlate with the experience of the pathologist. (6) There is a risk of complications arising from liver biopsy, and they can vary from mild symptoms, such as mild abdominal pain, to severe hemorrhage and injury to the biliary system. (7) Due to the risk of complications, some patients may refuse liver biopsy. (8) In hospital observation for 4–6 hours is usually required after liver biopsy. Furthermore, the use of ultrasound or the development of complications increases the cost of treatment and may also prolong hospitalization [6, 7, 12, 13, 18, 20–22].

2.2. The Importance of Noninvasive Markers of Liver Fibrosis. NIMs are helpful in assessing the stage of fibrosis in patients

with no clear indication for a liver biopsy, such as patients with chronic hepatitis B (CHB) and persistently normal serum alanine aminotransferase (ALT), patients with chronic hepatitis C (CHC) or CHB and who require follow-up assessment of the stage of fibrosis during or after treatment [13, 23], and autoimmune hepatitis (AIH) patients who require assessment after prolonged immunosuppressive therapy [24]. The rapid development of new medications for the treatment of some liver diseases, such as CHB, CHC, and nonalcoholic fatty liver disease (NAFLD), increases the requirement for more frequent evaluation of liver fibrosis to assess treatment response. Liver biopsies are not ideal for frequent evaluations.

The ideal NIM for assessing hepatic fibrosis must be simple, readily available, reliable, inexpensive, safe, and well validated in different forms of chronic liver disease. It must also be useful in assessing the progression of liver disease [7, 12, 13].

2.3. Mechanisms of Liver Injury That Result in the Production of Biomarkers.

The typical mechanism underlying the development of hepatic fibrosis is an imbalance between the deposition and removal of extracellular matrix (ECM). Hepatic stellate cells are the predominant producers of ECM, and their activation and proliferation are mediated by different cytokines during the process of liver injury [7, 8, 15, 25]. The activation and proliferation of Hepatic stellate cells ultimately result in an excessive deposition of ECM [7, 12, 25, 26]. In advanced fibrosis, the ECM may increase sixfold compared with that in normal liver [8, 26].

2.4. Noninvasive Biomarkers (NIBMs) for Assessing Liver Fibrosis

2.4.1. Classification of NIBMs for Liver Fibrosis.
NIBMs for liver fibrosis are grouped into two main categories: class 1 fibrosis markers, or direct biomarkers, and class 2 fibrosis markers, or indirect biomarkers [7, 12, 13, 15, 18]. The direct markers directly correlate with or are parts of the liver matrix produced by the Hepatic stellate cells during ECM turnover in the fibrosis process [7, 15, 18, 27]. In contrast, the indirect markers reflect changes in liver functions and are molecules released into the blood due to liver inflammation, but they do not correlate with ECM turnover [7, 27].

(a) Direct NIBMs

(i) Direct Markers Linked to Matrix Deposition
(1) Procollagen type 1 and type III.

Procollagen is a collagen precursor. It is cleaved by two different enzymes at its carboxy-terminal (type 1 (PC1CP)) and amino-terminal (type III (PCIIINP)), leading to the production of collagen. Mature collagen integrates into ECM [7, 8, 12, 15].

(I) The PCICP terminal peptide is major component of the connective tissue [7]. It has a higher upper limit of normal limit in male compared to females 202 μg and 170 μg, respectively [28]. It is normal in patients

with mild liver disease but increases in patients with moderate to severe cirrhosis [28–30].

(II) PCIIINP or PIIINP is another major component of connective tissue that has been extensively studied. Serum levels of PCIIINP reflect the stage of hepatic fibrosis [31–33]. During cirrhosis, PCIIINP serum levels correlate with serum bilirubin. An upper limit of normal for PIIINP was defined by Gallorini et al. as 0.8 U/mL [28]. Available data on PCIIINP in CHC and ALD show that it is increased and that its levels correlate with the severity of liver disease [30, 33–35]. Furthermore, a reduction in PCIIINP correlates with the response of CHC patients to treatment with interferon [28, 36]. The main limitation of using PCIIINP as a NIBM is that it is not specific to hepatic fibrosis and that it can be detected in other conditions. Furthermore, it shows lower efficacy compared with type IV collagen and hyaluronic acid [12, 29, 32]. More than a decade ago, PIIINP was evaluated in PBC and thought to be associated with histological severity of liver disease [37]. Similarly, McCullough et al. showed that PIIINP levels are increased in patients with AIH and that levels decrease in patients who respond to immunosuppressant treatment [38].

(2) Type IV collagen is a component of ECM that was investigated as a surrogate marker of liver fibrosis [13]. It has three different regions (an amino-terminal domain, a central helix domain, and a carboxy-terminal domain) [12]. Type IV collagen has been studied extensively in liver diseases of different etiologies [39]. It is increased in patients with liver diseases and its levels correlate significantly with the extent of hepatic fibrosis [36, 40–42]. At a cutoff level of ≥5.0 ng/mL type VI collagen had an AUC of 0.82 and NPV of 83,6% for the detection of severe fibrosis in NAFLD [41]. Walsh et al. had shown that type VI collagen is elevated in hepatitis C patients compared to control (median of 127.1 ng/mL, range 17.7–317.4 and median 61.3 ng/mL, range 11.5–102.3), respectively [42].

(3) Hyaluronic acid (HA) is a glycosaminoglycan, and it is a component of the ECM that is produced by Hepatic stellate cells [7, 36, 43]. An upper limit of normal range was defined by Gallorini et al. as 98 μg/L [28]. Variable cutoff point had been defined by different authors; Sakugawa et al. and Murawaki et al. had defined a cutoff level of ≥50 ng/mL for detection of severe fibrosis; in another study Montazeri et al. used a cut off level of 126.4 ng/mL [41]. HA has been studied in CHC, NAFLD, alcoholic liver disease (ALD), and CHB, but it has been more extensively studied in the former two diseases. HA has been of great value in detecting advanced fibrosis [36, 41, 44–46]. HA shows a negative predictive value of 98–100% for cirrhosis and is of great value in excluding cirrhosis [47–50]. In treated CHC patients, the response to treatment was reported to be associated with a reduction in serum HA levels [51–53].

(4) Laminin is a noncollagenous glycoprotein component of the ECM that is produced by hepatic stellate cells. It is deposited in the basement membrane of the liver [7, 8]. Serum levels of laminin are elevated above the upper limit

of normal range (0.59–1.4 U/mL) or (9.74–2.46) as defined by different authors [28, 41]. Furthermore Kropf et al. had proposed a cutoff value of 1.45 for laminin for the detection of both liver fibrosis and cirrhosis [54] in patients with chronic liver disease, and they correlate with the degree of perisinusoidal fibrosis [28, 33, 55, 56]. It showed an accuracy of 77% in the detection of significant fibrosis in CHC [13, 36]. Laminin has also been found to be of prognostic value, with a diagnostic accuracy of 70% for predicting the risk of variceal bleeding [55]. The data on basement membrane related to direct noninvasive markers showed that PICP, PIIINP, type VI collagen, and laminin levels decrease during abstinence from alcohol intake [36, 55, 57].

YXL-40 chondrex is a member of the chitinase family, and it is involved in the remodeling and degradation of the ECM [58]. Increased serum levels of YXL-40 chondrex to (330 μg/L), have been shown to correlate with the degree of fibrosis in all forms of liver disease and similar observations were made for cirrhosis (425 μg/L) as compared to age matched normal controls (102 μg/L) [58, 59]. Saitou et al. had defined different cutoff levels for fibrosis and cirrhosis 186.4 and 284.8 μg/L, respectively [59]. YXL-40 levels have also been observed to correlate with HA levels [58, 59]. The serum level of YXL-40 during postinterferon therapy for CHC significantly decreased in both responder and nonresponder patients [59].

(ii) Direct Markers Linked to Matrix Degradation. Degradation of the EMC is an action primarily of the family of metalloproteinase enzymes (MMPs), three of which are expressed in humans [60].

(1) MMP-1 (collagenases): Murawaki et al. showed that the levels of MMP-1 are inversely correlated with histological severity, including both necrosis and fibrosis. However, in contrast, MMP-1/TIMP-1 (tissue inhibitors of matrix metalloproteinases) complex levels correlate with the degree of portal inflammation but not with the extent of hepatic fibrosis [61].

(2) MMP-2 (gelatinase-A): MMP-2 is secreted by hepatic stellate cells during liver disease, but data on its role in the staging of fibrosis have been variable. There is currently no clear association of MMP-2 with hepatic fibrosis [62, 63], but Boeker et al. showed that it has a high diagnostic accuracy of 92% for detecting cirrhosis secondary to CHC [62]. A cutoff value of 0.550 was defined by Murawaki et al. and higher levels were associated with severe fibrosis [47], but Boeker et al. had shown that the cutoff value will be changed according to the method that has been used for measuring MMP-2. However they showed that cirrhotic patients have 2.4-fold elevation of MMP-2 compared to controls [62].

(3) MMP-9 (gelatinase-B): a product of hepatic Kupffer cells, MMP-9 was previously thought to be of value in the diagnosis of hepatocellular carcinoma [64]. Recently, Badra et al. showed that MMP-9 correlated negatively with both TIMP-1 and histological severity in chronic hepatitis, with the lowest levels detected in patients with cirrhosis [64, 65].

(4) Tissue inhibitors of matrix metalloproteinases (TIMPs): these proteins interfere with MMP functions and lead to the inhibition of ECM degeneration. TIMP-1 interacts with most MMPs, and TIMP-2 interacts specifically with

MMP-2. Boeker et al. had shown that serum levels of TIMP-1 increase 2.4 times in patients with cirrhosis compared to controls [7, 8, 15]. The serum levels of TIMPs increase with the progression of liver disease and directly correlate with fibrotic stage [62–67].

(iii) Cytokines and Chemokines Linked to Liver Fibrosis
(1) Transforming growth factor-β (TGF-β1) is the most important stimulus for ECM deposition. It has pleiotropic effects via membrane receptors on cells [68]. TGF-β levels were higher in hepatitis C virus infected patient and they were found to correlate with the progression of hepatic fibrosis [69, 70]. A level less than 75 ng/mL was predictive of stable disease [69].

(2) Transforming growth factor alpha (TGF-α) was found to enhance the proliferation of hepatic stellate cells by inducing the entry of hepatic stellate cells into S-phase. In patients with liver disease, TGF-α was found to correlate with the progression of liver disease, Child-Pugh classification, and it is increased in patients with HCC [71]. 3-Platelet-derived growth factor (PDGF) is the most potent mitogen of hepatic stellate cells *in vitro* [15, 72]. Studies on the role of PDGF in liver fibrosis have shown that its levels correlate with the severity of hepatic fibrosis [73, 74]. Zhang et al. had shown that PDGF at a cutoff value of 40.50 ng/L strongly correlates with the stage of fibrosis and inflammation [73].

(b) Indirect Biochemical Markers of Hepatic Fibrosis
(1) Serum alanine aminotransferase (ALT) is one of the oldest markers used to assess liver disease [12]. Pradat et al. have shown that serum ALT is beneficial to measure due to its high sensitivity and specificity (2.25-fold greater than the normal levels predicts liver histology) [75]. However, serum ALT levels are affected by many factors, including gender, body mass index, and the use of hepatotoxic medications [76, 77].

(2) The aspartate aminotransferase (AST)/ALT (AAR) ratio is one of the eldest markers of liver fibrosis that is easily available and applicable. It has been validated in different forms of liver disease, [78, 79] and a ratio of >1 is predictive of cirrhosis [80, 81]. An AAR of 1.16 has been found to predict one-year mortality with high accuracy [80]. The BARD score includes the AAR together with the BMI and diabetes measurements and was proposed by Harrison et al. in 2008. It showed NPVs of 96% and 81.3% and showed an enhanced performance compared with the NFAS [82, 83].

(3) The AST/platelet ratio (APRI) was developed by Wai et al. in 2003 [84] and is measured as APRI = AS level (/ULN) H100/platelet count [84]. In the original study, the APRI of more than 1.5 showed an area under the receiver operating curve (AUC in the ROC) of 0.8, and showed an area under the receiver operating curve (AUC in the ROC) of 0.8, and a 0.89 for advanced fibrosis F3-F4 and cirrhosis respectively [84]. Several other studies have been conducted to validate the APRI [12, 13]. Multiple studies had shown that it is of great value and has high accuracy in predicting advanced fibrosis in different forms of liver disease [85–88]. Snyder et al. had shown that APRI at a cutoff 0.42 or less correctly detected mild fibrosis with a NPV of 95% [89]. In contrast, some

studies showed that the APRI is only of moderate accuracy in assessing fibrosis in CHC [90]. Loaeza-del-Castillo et al. demonstrated that the APRI is not of diagnostic value in assessing fibrosis in autoimmune hepatitis (AIH) patients. Furthermore, in the same study, the authors showed that this ratio was capable of predicting significant fibrosis in both CHC and NAFLD patients [79, 87]. Chrysanthos et al. showed that when using the APRI alone, the stage of fibrosis is incorrectly classified in 40–65% of patients [91]. However Snyder et al. had shown that adding the FIBROSpect II to APRI will correctly classify hepatic fibrosis in additional patients and will lower the indeterminate zone to 25.8% [89]. The diagnostic accuracy of APRI was improved by Lok et al. by the incorporation of ALT and the international normalized ratio (INR) [92]. Furthermore, the APRI was also found to be of high diagnostic accuracy in assessing the progression of fibrosis in postliver transplant patients [93].

(4) The Forns index: this index was described by Forns et al. in 2002. It is calculated based on the age of the patient and three routine laboratory tests, namely, platelet count, cholesterol level, and γ glutamyl transferase (GGT) [94]. At a cut of value of 6.9, it was noted to be of value in differentiating mild fibrosis (F0-F1) from severe fibrosis (F2–F4), but it is less accurate in the differentiation of F2 from F4 [7]. Similar to the APRI, the Forns index may misclassify half of a patient population [13, 85, 94].

(5) The PGA index was proposed by Poynard et al. in 1977 as a marker to assess alcoholic liver disease. It is generated via a combination of GGT, the prothrombin index, and apolipoprotein A [95]. This index was additionally modified by including α_2 macroglobulin (PGAA) as a contributing factor, which increased its accuracy from 65% for PGA to 70% for PGAA [96].

(6) Fibro test and Fibrosure: these tests are identical but are marketed under different names [7]. The test is conducted based on the patient age, gender, serum haptoglobin, α_2 macroglobulin, apolipoprotein A1, GGT, and bilirubin [97, 98]. Variable ranges of Fibro test had been obtained according to the stage of fibrosis; a result of 0.75–1 and 0.73–0.74 was obtained for stage F4 and stages F3-F4, respectively [97]. The accuracy of the Fibro test has been assessed in CHC, CHB, NAFLD, and ALD patients. It is the most validated noninvasive test used to detect hepatic fibrosis [7, 13, 99–101]. The Fibro test may be less useful for the detection of intermediate stages of fibrosis (F2) compared with the extreme stages of F0-1 and F4 [12]. Recently, Poynard et al. confirmed the accuracy of the Fibro test in the diagnosis of advanced fibrosis and cirrhosis. That study included 1289 patients with CHC and 604 controls. The specificity/sensitivity for advanced fibrosis was 0.93/0.70 and, in the case cirrhosis, the specificity/sensitivity was 0.87/0.41 [102]. In a study of patients with severe obesity, Poynard et al. demonstrated high accuracy of the Fibro test in diagnosing cases of steatohepatitis, with an AUC of 0.85 [103]. Furthermore, and in a more recent publication on the Fibro test, Poynard et al. validated the use of the Fibro test during follow-up to monitor the progression of the most frequent forms of chronic liver disease [104].

ACTI test: the Acti test is a modification of the Fibro test in which ALT values are added. It reflects both necroinflammatory activity and liver fibrosis [103, 105]. Sebastiani et al. revealed that the Acti test showed a negative predictive value of 0.36 for excluding significant necrosis (85%) [85]. Together with the Fibro test, the Acti test may help assess both fibrosis and necrosis, and both tests may be reliable alternatives to liver biopsies [106].

(7) The Fibro index: this index was developed in 2007 by Koda et al. to assess hepatic fibrosis in CHC [107]. It is obtained from the platelet count, AST, and gamma globulin values. At a cutoff value of 2.25 it was associated with F2-F3 fibrosis and NPV of 90% [107]. This index showed an AUC of 0.83 for the detection of significant fibrosis [107]; however, subsequent validations have shown this index to be less robust [108].

(8) The FIB-4 score: This score is calculated based on age, platelet count, AST, and ALT. It was first developed by Sterling et al. to assess fibrosis in HIV/HCV coinfected patients at a cutoff value of 3.25; 87% of patients were correctly classified, with an AUC of 0.765 for significant fibrosis [109]. The Fib-4 score was subsequently validated for detection of the monoinfections HCV and HBV. It showed AUCs of 0.85 and 0.81 for the detection of severe fibrosis, for isolated HCV and HBV infection, respectively [110, 111]. Fib-4 showed a better performance in NAFLD compared with the AAR, APRI, and NAFLD fibrosis score (NFSA) [79, 112].

(9) The FibroQ test: this test was proposed by Hsieh et al. in 2009. It is calculated based on age, AST, prothrombin time (PT-INR), platelet count, and ALT [113]. In that study, using a cutoff value of 1.6 the AUC for the detection of significant fibrosis was 0.783, and the negative predictive value was 100% for the exclusion of cirrhosis. These values were both higher than those obtained when using the APRI and AAR in the same cohort [113, 114]. More recently, a similar study showed that FibroQ was superior to FIB-4, AAR, APRI, and Lok's model in predicting significant fibrosis in patients with chronic hepatitis C [113, 114].

(10) Currently, with the increase in the incidence of metabolic syndromes, NAFLD is considered the most frequent cause of liver disease in the world [115]. NAFLD specific markers for fibrosis have been developed. The simple test was proposed to assess the stage of hepatic fibrosis in NAFLD. The test is based on body mass index, age, glycemic status, platelet count, albumin level, and the AST/ALT ratio [116]. Using this test, 90% of patients were correctly staged, with AUCs of 0.88 and 0.82 in the two groups that were studied, and advanced fibrosis was excluded with high accuracy (NPV of 93% and 88% in the two groups) [116].

(11) Steato test: this test was proposed by Poynard et al. to assess NAFLD. It incorporates the five components of the Fibro test (α_2 macroglobulin, haptoglobin, apolipoprotein A1, GGT, and total bilirubin) and the Acti Test (ALT in addition to body mass index, serum cholesterol, triglycerides, and glucose, adjusted for age and gender). A cutoff value of 0.7 resulted in a 90% specificity, permitting the authors to achieve NPV and PPV values of 93% and 63%, respectively, with a steatosis prevalence of 30% [117]. The AUCs ranged from 0.72 to 0.86 for the three validation groups in that

study, [117] and similar result was obtained by Poynard et al. [103]. Furthermore, Poynard et al. proposed other algorithms that combined 13 parameters, including age, gender, height, weight, and serum levels of triglycerides, cholesterol, α_2 macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyltranspeptidase, transaminases, ALT, AST, and total bilirubin. Using this algorithm at a value of 0.75, they obtained an AUC of 0.79 for the diagnosis of NASH in the validation group and an AUC of 0.83 for a diagnosis of no NASH in the same group [118]. The Nash test has also been validated in combination with other tests, including the Fibro test and the Steato test [119].

(c) Combined Direct and Indirect Markers

(1) The Fibrometer test was described by Calès et al. in 2005. It is performed by combining the platelet count, prothrombin index, aspartate aminotransferase, α_2-macroglobulin (A2M), hyaluronate, urea, and age. The test results indicate the amount of hepatic fibrosis as a percent of fibrous tissue within the liver [120]. The test has been validated in viral hepatitis and ALD and demonstrates AUCs of 0.883 and 0.962, respectively, for the detection of advanced fibrosis at stages F2–F4 [120]. The Fibrometer has also been validated by the same author in NAFLD, with a reported AUC of 0.943 [121]. When compared to other indirect tests the Fibrometer showed an AUC of 0.892 for detecting stage F2–F4 fibrosis in CHC and CHB. This value was higher than those obtained for the Fibro test, Forns index, and APRI, which were 0.808, 0.82, and 0.794, respectively [121]. Similarly the same study showed that Fibrometer in NAFLD performed better than NFSA, with AUCs of 0.943 and 0.884, for both tests, respectively, for detecting significant fibrosis [121].

(2) Fibrospect II test combines three parameters: hyaluronic acid, TIMP-1, and α_2 macroglobulin. At a cutoff value of 42, it can differentiate mild F0-F1 from severe fibrosisF2–F4 [122]. It was validated in CHC patients, and it showed an AUC of 0.831 for the detection of significant fibrosis at stages F2–F4 [123]. Furthermore a similar AUC 0.83 for detection of advanced fibrosis F2–F4 was obtained by Jeffers et al. in a study of 145 CHB and CHC patients [124]. Subsequent similar studies using Fibrospect II showed higher AUC [99, 125].

(3) SHASTA index is based on serum hyaluronic acid, AST, and albumin. In a study of 95 HIV/HCV coinfected patients, an index of 0.3 showed a sensitivity of >88% and a negative predictive value of >94%, and a level of 0.8 showed a specificity of 100% and a positive predictive value of 100% for detection of severe fibrosis of F3 or more [126]. Using this index only, 42% of patients were correctly classified, whereas the remaining 58% showed values between 0.3 and 0.8 [126].

(4) The Hepascore model was proposed by Adams et al. in 2005. It combines age, gender, serum bilirubin, GGT, hyaluronic acid, and α_2 macroglobulin. At a cutoff value of 0.5, it showed AUCs of 0.82, 0.9, and 0.89 for the detection of significant fibrosis, advanced fibrosis, and cirrhosis, respectively, in CHC [127]. More recently, Guéchot et al. showed similar findings when using the automated Hepascore [128],

which has been validated in patients with ALD and showed AUC similar to that of Fibro test, Fibrometer for detection of advanced fibrosis with an AUC of 0.83 ± 0.03 [129].

(5) European liver fibrosis panel (ELF) test was proposed by the ELF panel [13, 130]. Its calculation is based on age, hyaluronic acid, amino-terminal properties of type III collagen (PIIINP), and the tissue inhibitor of matrix metalloproteinase 1. In the original calculation, age was included and the value was called the OELF [130], but the calculation was then simplified to a set of parameters that did not include age. The sensitivity of ELF for the detection of stage 3 or 4 fibrosis was 90%. ELF at a result of more than 0.102 showed a negative predictive value for significant fibrosis F3-F4 of 92% and an AUC of 0.804 [130]. The ELF has been found to be of value for assessing fibrosis in chronic viral hepatitis, autoimmune liver disease, ALD, and NAFLD because the AUC in different studies has ranged from 0.773 for CHC to 0.98 for NAFLD [45, 130, 131].

2.5. Noninvasive Markers That Are Less Commonly Studied and Validated for the Assessment of Liver Fibrosis

(1) ^{13}C-methacetin breath test (MBT) and ^{13}C-caffeine breath test (CBT) are tests that assess cytochrome P450-dependent hepatocellular function [132, 133]. ^{13}C-methacetin is metabolized by healthy liver into acetaminophen and $^{13}CO_2$. An increase in breath levels of $^{13}CO_2$ may be measured using mass spectrometry or infrared spectroscopy. Dinesen et al. showed that the MBT had AUCs of 0.827 and 0.958 for the detection of advanced fibrosis and cirrhosis, respectively [134]. Similarly, caffeine undergoes extensive hepatic metabolism, principally via demethylation by cytochrome P450. This metabolism results in the production of CO_2. Cirrhotic patients show reduced caffeine metabolism that results in significantly lower levels of $^{13}CO_2$ compared with those of control individuals when ^{13}C-caffeine is administered orally [134]. A significant inverse relationship exists between the CBT and Child-Pugh score ($P = 0.002$) [134].

(2) Proteomics and glycomics: proteins and glycoproteins are assessed using mass spectrometry. Using serum samples [135], initial proteomics and glycomics studies of liver fibrosis showed promising results [136, 137]. However, more recent data have shown that these methods are of limited value for the assessment of liver fibrosis [138].

(3) Kam et al. proposed the Fibro-Glyco index for assessing liver fibrosis; it is based on the N-glycome level determined using mass spectrometry. They reported a significant correlation between this index and the degrees of liver fibrosis ($r = 0.784$, $P = 0.01$). In addition, the index is useful in the detection of liver fibrosis and cirrhosis, with an ROC of 0.91 for both [139].

(4) King's score: this score is the most recently proposed noninvasive index [140]. It is calculated using the formula ks = Age (years) $\times$ AST (IU/L) $\times$ INR/Platelets $\times 10^9$/L. It shows AUCs for detecting advanced fibrosis and cirrhosis of 0.82 and 0.89, respectively [140].

(5) Noninvasive hepatitis C-related cirrhosis early detection (NIHCED) index was suggested by Bejarano-Redondo

et al. in 2009 for the detection of F2–F4 fibrosis. It is assessed based on age (≥60 years), prothrombin time (≥1.1), platelets (≤100,000), and AST/ALT (≥1). In addition to the presence of right hepatic lobe atrophy, caudate lobe hypertrophy is observed upon ultrasound examination. This test at score of more than 6 shows an accuracy of 72% and an AUC of 0.787 [141].

(6) Two Chinese models that involve different NIBMs have been suggested for assessing CHB [142, 143]. In the first model, Liu et al. used haptoglobin, GGT, and platelet counts, and their model was of high diagnostic value in assessing patients with HBeAg-positive and HBeAg-negative CHB [142]. In the second model, Tu et al. used APRI, GGT, INR, and HBeAg. The model was effective in differentiating early and advanced fibrosis and active cirrhosis [143].

(7) More recently, data on the use of surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-ATAOF-MS) in HCC identified a panel of serum proteins of value in differentiating HCC patients from those with cirrhosis or normal controls [144, 145].

2.6. Viral Hepatitis/HIV Coinfected Patients. As mentioned above SHASTA index was used for HIV/CHC coinfected patients [126]. The FIB-4 index was found to be superior to APRI in the diagnosis of mild from severe fibrosis in HIV/CHC coinfected patients [146]. Furthermore, Bottero et al. evaluated the use of different indirect noninvasive markers for assessing liver fibrosis in HBV/HIV coinfected patients. Based on the AUC, they concluded that the Fibrometer, Hepascore, and Zeng's score were the most accurate noninvasive biochemical scores for assessing liver fibrosis for HIV/HBV coinfection. In addition, the performance of the biomarkers was not significantly improved by combining two biochemical scores [147].

3. Combination of Markers

Several authors have attempted to combine NIMs to assess hepatic fibrosis, and they have suggested that these combinations improved sensitivity. In 2006, Sebastiani et al. proposed the SAFE algorithm (sequential algorithm for fibrosis evaluation) for use in CHC patients. In that study, 190 CHC patients were assessed using the APRI, Forns index, and Fibro test at the time of liver biopsy. The authors observed that the optimal combination was APRI followed by the Fibro test. Using this algorithm, advanced fibrosis and cirrhosis were diagnosed with accuracies >94% and 95%, respectively, and the requirement for liver biopsy was reduced by 60–70% [85]. The same group had previously validated the SAFE biopsy algorithm in a larger number of patients (2035). They demonstrated accuracies of 90.1% and 92.5% for detecting advanced fibrosis and cirrhosis, respectively [148]. Furthermore, Castéra et al., in another study of 314 CHC patients recently compared the SAFE biopsy with the Castéra algorithm (combination of transient elastography and Fibro test) and demonstrated that the Castéra algorithm prevented more liver biopsies than the SAFE biopsy in cases of significant fibrosis, but it showed reduced accuracy (87.7% versus 97%). In contrast, the accuracy of the Castéra algorithm for diagnosing cirrhosis was greater than that of the SAFE algorithm (95.7% versus 88.7%) [149]. Similarly, Sebastiani et al. evaluated a stepwise combination algorithm that included the APRI, Fibro test, and liver biopsy for the diagnosis of CHB. They demonstrated AUCs of 0.96 and 0.95 for the detection of significant fibrosis and cirrhosis, respectively, with a 50–80% reduction in the requirement for liver biopsy [150]. The Fibropaca algorithm had been proposed by Bourliere et al. in 2006, and it involved combining the Fibro test, APRI, and Forns index for the diagnosis of 235 CHC patients. Using this algorithm, 81.3% of patients were correctly diagnosed, and only 18.7% of patients required a liver biopsy [151]. Leroy et al. evaluated the performance of different combinations of NIM for assessing hepatic fibrosis in 180 CHC patients: MP3 score (combination of PIIINP and MMP-1), FT, Frons index, Hepascore, Fibrometer, and APRI. They noted that MP3 and APRI were the only independent variables associated with significant fibrosis [152]. In that study, the optimum combination was reliable in 1/3 of patients [152]. In another study, Bourlier et al. used different stepwise combinations of the Hepascore, Fibro test, APRI, and Forns index; they reported that the SAFE biopsy algorithm proposed by Sabastiani et al. showed an accuracy of 90% in CHC patients, and biopsy was required in only 44% of patients [153]. In the same study, when the APRI was used as a screening tool followed by the Hepascore, liver biopsy was avoided in 45% of patients [153].

Several studies were conducted that compared different algorithms that combined direct NIBMs. The majority of studies showed comparable results for different combinations of NIBMs. Furthermore, the combination algorithms showed significantly better performances compared with individual markers [49]. A combination algorithm of different direct noninvasive markers was proposed by Patel et al. In that study, hyaluronic acid, TIMP-1, and α_2-macroglobulin were combined for the assessment of fibrosis in CHC patients, and an accuracy of 75% was demonstrated for the detection of F2–F4 fibrosis [154].

4. Comparison of Algorithms Incorporating Different Indirect NIBMs

Several studies have compared the accuracies of different NIBMs for detecting advanced fibrosis and cirrhosis. In Bourliere's study, the Fibro test and Hepascore showed similar diagnostic profiles for fibrosis of stages F2–F4 [153]. Similarly, Sebastiani et al., using another combination algorithm, showed that the Fibro test was more accurate compared with both the APRI and Forns index [85]. In the study of Lackner et al., the APRI showed greater accuracy than the AAR for the detection of both advanced fibrosis and cirrhosis in CHC patients ($P < 0.05$) [155]. More recently, in a study of both CHC and CHB patients with postresection hepatocellular carcinoma, Lin et al. confirmed the superiority of APRI over AAR in the detection of both advanced fibrosis and cirrhosis [156]. In their study of different combinations of NIBMs for assessing fibrosis in CHC using six noninvasive tests, Leroy et al. demonstrated that the Fibrometer showed the

TABLE 1: AUROC for the direct markers that have been used in assessment of fibrosis in various liver diseases.

Marker	Liver disease evaluated by the markers				AURCO for advanced fibrosis	AURCO for cirrhosis	References
	CHC	CHB	NAFLD	ALD			
PCICP	NA	—	—	NA	NA	NA	[28–30, 82]
PCIIINP	0.69–0.78	—	NA	0.67–0.867	0.67–0.867	0.734	[31, 36, 37, 58, 154, 159]
Type IV collagen	0.73–0.83	—	0.82	NA	0.583–0.83	NA	[30, 41, 42, 58, 154, 159]
HA*	0.821–0.92	0.98	0.97	0.69–0.93	0.69–0.98	0.85–0.93	[31, 32, 41, 44, 46–48, 115, 154, 159–162]
Laminin	0.542–0.82	—	NA	NA	0.46–0.82	NA	[30, 33, 42, 56, 159]
YXL-40	0.7–0.81		NA	NA	0.7–0.81	0.79	[57–59, 154]
MMP-2	0.59				0.59	0.97	[47, 62]
MMP-3	0.88	—	—	—	0.88	NA	[152]
MMP-9	**	—	—	—	NA	NA	[64, 65]
TIMP-1	0.71–0.773	—	—	—	0.68–0.73	0.91	[31, 62, 63, 154]
TIMP-2	0.73	—	—	—	0.73	NA	[63]
TGF-β1	NA	—	—	—	—	—	[70, 71]
TGF-α	NA	—	—	—	—	—	[70, 71]
PDGF	NA	—	—	—	—	—	[72, 73]

*HA: Hyaluronic acid.
**Negative association, MMP-9 decreases with the progression of fibrosis.
NA: Area under receiver operating characteristic (AUROC) is not available.

best performance, followed by the Fibro test, with AUCs of 0.86 and 0.84, respectively. In contrast, the Forns index and Hepascore showed the lowest performance, with AUCs of 0.78 and 0.79, respectively [152]. The Fibro test was also found to be more accurate and cost-effective compared with Fibrospect II in testing liver fibrosis in HCV genotype 1 patients [157].

4.1. The Role of NIBMs in Assessing the Development of Varices in Liver Disease. Stefanescu et al. recently validated the guidelines for the use of NIBMs in detecting large varices compared with endoscopy. They used the APRI, FIB-4, Forns index, and Lok score in addition to the Fibroscan. They concluded that a combination of the Lok score and the Fibroscan was optimal for detecting large varices [158].

Summary of NIBMs for assessment of different forms of liver disease (Tables 1, 2, 3, and 4) [159–170], CHC was the first and most extensively studied liver disease with respect to the utilization of different NIBMs, but NIBMs have been evaluated much less in CHB compared to CHC. Different studies have used different NIBM cutoffs levels for detecting advanced fibrosis and/or cirrhosis for the same liver diseases. The results demonstrated differences in the sensitivity, specificity, and accuracy of the same markers or scores. Another possible reason underlying the differences in the results may be the selection bias in the study populations of different studies. For example, a cohort study that includes more patients with advanced liver fibrosis will show different NIBM results compared with a study that utilizes a cohort in which fewer patients have advanced liver fibrosis.

4.2. Pros and Cons of NIBMs for Detecting Liver Fibrosis. NIBMs are advantageous compared with liver biopsies because of the following reasons.

(1) They are noninvasive and can be measured in outpatient departments.

(2) They cost less compared with liver biopsies.

(3) They can be easily repeated for confirmation.

(4) If they are well validated, they may be used for follow-up and monitoring in the future.

(5) They are not associated with the liver biopsy morbidity and mortality risks.

Limitations of NIBMs:

(1) Some of markers like APRI, Hepascore, and Fibrospect II need more validation in intermediate stages of liver fibrosis [99].

   (1) In spite that the effectiveness of NIBM in assessment of liver fibrosis was demonstrated by many studies some studies had shown that they may not be of diagnostic value in the detection of liver fibrosis [171].

   (2) They remain of limited value in assessing the development of complications, like esophageal varices and chance of variceal bleeding [99].

   (3) Both direct and indirect markers of liver fibrosis are not liver-specific and can be altered by pathological conditions in other organs.

   (4) Some of the biomarkers lack standardization due to variable values and the different upper-normal ranges used by different laboratories.

   (5) All studies that evaluated the accuracy of NIBMs used the liver biopsy as the gold standard reference; this protocol is also a limitation because even the best liver biopsy retains a risk of sampling error.

TABLE 2: AUROC for the indirect markers that have been used in assessment of fibrosis in various liver diseases.

Marker	Liver disease evaluated by various markers				AURCO for advanced fibrosis	AURCO for cirrhosis	References
	CHC	CHB	NAFLD	ALD			
ALT (2.25) the normal	0.716–0.815				0.716–0.815	—	[75, 163]
AST/ALT ratio	0.54–0.709	NA	0.742–0.83	NA	0.54–0.83	0.67	[78, 80, 112, 114, 155, 162, 164]
APRI	0.65–0.87	0.67–0.72	0.564–0.866	—	0.564–0.87	0.75–0.92	[79, 87–91, 112, 120, 150, 156, 164–167]
Frons index	0.78–0.86	NA	—	—	0.78–0.86		[94, 152]
PGAA index	—	—	—	NA	—	—	[95, 96]
Fibro test	0.72–0.87	0.76–0.85	0.82–0.89	0.83–0.91	0.72–0.87	0.77–0.94	[99–101, 104–106, 112, 120, 129, 152, 153, 159, 169, 170]
Acti test	NA		0.77 SH				[97, 105, 106]
Fibro index	0.804–0.83-	NA	—	—	0.82	0.845	[107, 108, 120, 164]
Fib-4*	0.785–0.86	0.81	—	—	0.785–0.86	—	[109–111, 114, 166]
FibroQ	0.789	NA NA	—	—	0.783	0.791	[113, 114]
The simple test (NAFLD) fibrosis score	—	—	0.82–0.89	—	0.82–0.89	—	[116, 121, 131]
Steato test	—	—	0.799–0.86		0.799–0.86	—	[117]
SELDI-TOF protein chip	0.88	0.926	—	—	0.88–0.906	0.921	[137, 170]
13C metacetin breath test	0.83-	—	—	—	0.83	0.96	[133, 164]

*Used to assess fibrosis in HCV/HIV coinfected patients.
NA: studied but AUROC is not available.

TABLE 3: AUROC for liver fibrosis biomarkers that are a mix of direct and indirect markers.

Marker	Liver disease evaluated by various markers				AURCO for advanced fibrosis	AURCO for cirrhosis	References
	CHC	CHB	NAFLD	ALD			
The Fibrometer	0.892**		0.943	0.83–0.962	0.883–0.962	0.94	[120, 121, 129, 152]
Fibrospect II	0.77–0.831	NA	—	0.83	0.77–0.831		[122, 124, 125]
SHASTA index***	0.878	—	—	—	0.878	—	[126]
Hepascore	0.82			0.83	0.82–0.9	0.89–0.92	[127, 129, 152, 153]
ELF	0.773	0.93–0.98	0.873	0.944	0.773–0.98		[130, 131]#
		—		—			

**mixed population of 337 HCV and 46 HBV patients.
***HVC and HIV co-infected patients.
#1021 subjects recruited; the numbers in each diagnostic category were as follows: chronic hepatitis C, 496; ALD, 64; fatty liver, 61; hepatitis B, 61; primary biliary cirrhosis or primary sclerosing cholangitis, 53; recurrent disease after liver transplantation, 48; autoimmune hepatitis, 45; hemochromatosis, 32; cryptogenic cirrhosis, 19; hepatitis B and C, 4; 138 patients with other causes of liver disease like granuloma and abnormal liver enzymes from unknown cause.
NA: studied but AUROC is not available.

TABLE 4: AUROC for performance of combination algorithms in assessing liver fibrosis.

Combination algorithm	Liver disease evaluated by various markers				AURCO for advanced fibrosis	AURCO for cirrhosis	References
	CHC	CHB	NAFLD	ALD			
SAFE biopsy	0.89–1	—	—	—	0.97–1	0.87	[85, 148, 158]
Stepwise SAFE algorithm + biopsy	—	NA		—	0.96	0.95	
Castera	0.97	—	—	—	0.97	0.93	[149]
Bourliere algorithms	NA	—	—	—	—	—	[153]

NA: studied but AUROC is not available.

(6) A selection bias of the studied population may have biased the results; for example, if a larger number of patients with advanced or minimal fibrosis are included, this bias will affect the accuracy of the markers [18].

(7) In a large population of patients with liver diseases, for example, patients with autoimmune liver disease, NIBMs remain poorly evaluated and validated.

(8) The majority of the direct markers that have been evaluated are not routinely available in all laboratories.

Investigators must work to overcome the limitations of NIBMs for liver fibrosis. Several studies of marker combinations or stepwise algorithms have shown improved performance compared with the performance of individual markers [49]. Furthermore, the recent use of NIBMs together with transient elastography for assessing hepatic fibrosis has demonstrated improved outcome without requiring liver biopsy in most patients with viral hepatitis [19, 163].

4.3. Future Studies Using NIBMs Are Required

(1) NIBMs can be used to assess disease progression and to predict complications and survival of liver disease patients.

(2) NIBMs can be used to monitor treatment responses.

(3) Because of its complications, a liver biopsy cannot be used for the screening of high-risk groups, such as CHB patients with normal liver enzymes and obese or diabetic patients with expected NAFLD. Thus, NIBMs can be used to screen these patients.

(4) Proposing new combinations of direct and indirect markers may be a goal of future studies to avoid the limitations of each type of marker and to increase diagnostic accuracy.

(5) NIBMs for assessing liver fibrosis have not yet been validated in other less common liver diseases, such as AIH and PBC.

Considering the above-mentioned limitations and patients who fall in the gray areas using the noninvasive markers, liver biopsy is still required to diagnose some patients, for example, patients with viral hepatitis B or C and secondary diagnosis like AIH, NAFLD, or ALD [19]. Similarly, patients who have negative testing for viral markers and auto-antibodies, the possibility of NAFLD or autoantibody negative AIH can be supported or excluded by liver biopsy. Furthermore toxic liver damage like methotrexate induced liver injury had not been well evaluated using noninvasive markers.

5. Conclusion

Currently, a perfect NIBM for liver histology is unavailable. However, utilization of noninvasive biomarkers for liver histology can significantly reduce, but not completely replace, the requirement for liver biopsies in patients with chronic viral hepatitis and NAFLD. For the other types of liver disease, NIBMs are not well validated and more studies are required. Furthermore, future studies on the currently available NIBMs may reveal more important prognostic capabilities of these markers.

Abbreviations

NIBM: Noninvasive biomarkers
NAFLD: Nonalcoholic fatty liver disease
ALD: Alcoholic liver disease
ECM: Extracellular matrix
AUROC: Area under receiver operating characteristic.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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