Noninvasive Biomarkers of Liver Fibrosis: Clinical Applications and Future Directions

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Abstract Chronic liver disease is a significant cause of morbidity and mortality worldwide. Current strategies for assessing prognosis and treatment rely on accurate assessment of disease stage. Liver biopsy is the gold standard for assessing fibrosis stage but has many limitations. Noninvasive biomarkers of liver fibrosis have been extensively designed, studied, and validated in a variety of liver diseases. With the advent of direct acting antivirals and the rise in obesity-related liver disease, there is a growing need to establish these noninvasive methods in the clinic. In addition, it has become increasingly clear over the last few years that noninvasive biomarkers can also be used to monitor response to antifibrotic therapies and predict liver outcomes, including hepatocellular carcinoma development. This review highlights the most well-established noninvasive biomarkers to date, with a particular emphasis on serum and imaging-based methodologies.

Keywords Noninvasive · Liver · Fibrosis · Biomarkers · Serum · Molecular imaging

Introduction

Chronic liver disease is characterized by progressive fibrosis caused by repeated injury due either to metabolic dysfunction, alcohol abuse, viral hepatitis, or autoimmune disease. At the cellular level, an imbalance occurs between extracellular matrix (ECM) synthesis and degradation resulting in fibrosis and eventual cirrhosis of the liver. Accurate assessment of fibrosis stage and early detection of cirrhosis are vital for determining prognosis and guiding management, since doing so identifies those patients at greatest risk of developing complications of cirrhosis, including hepatocellular carcinoma (HCC) and esophageal varices, for which longitudinal survey is essential.

Liver Biopsy—An “Imperfect” Gold Standard

Biopsy has been the gold standard in diagnosing and staging liver fibrosis and also has the advantage of assessing other disease aspects as well including inflammation, steatosis, and necrosis. However, biopsy is often considered an “imperfect” gold standard, since it suffers from intra/inter-observer variability and is associated with several complications including hospitalization in 1–5 % of cases and mortality in 0.01–0.1 % of cases [1, 2]. In addition, biopsy is subject to sampling error as the 1–2 pieces of 1-cm-long tissue only accounts for 1/50,000 of the liver volume [3]. For example, laparoscopic liver biopsies from the right and left lobes of HCV patients differed by at least one Metavir stage in 33 % of cases [4], and higher sampling variability has been reported for

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NASH and biliary fibrosis [5, 6]. Sampling error even exists for advanced stages of disease as biopsy can lead to under-staging of cirrhosis in up to 20 % of patients [7]. Importantly, repeated biopsies to evaluate disease progression or response to treatment are impractical due to the increased risk of complications and poor patient compliance. For all of these reasons, noninvasive strategies that can repeatedly assess liver fibrosis throughout the entire organ are urgently needed to assess disease stage, monitor treatment response, and determine prognosis.

The Clinical Need for Noninvasive Biomarkers

The clinical need for accurate, noninvasive alternatives to liver biopsy is driven by the growing burden of chronic liver disease worldwide. Unlike other major causes of mortality, rates of chronic liver disease are increasing rather than declining [8]. Chronic liver disease results from a wide range of etiological factors including hepatitis B virus (HBV), hepatitis C virus (HCV), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD). In the US, over a million patients could be seeking care for HCV in the coming decade due to the introduction of effective anti-HCV therapies and also the recent CDC recommendation to screen all patients born between 1945 and 1965 for HCV infection [9•]. Furthermore, the prevalence of NAFLD in the US could be as high as 30–46 % [10, 11]. NAFLD is a spectrum of disease encompassing simple steatosis to nonalcoholic steatohepatitis (NASH). Compared to patients with steatosis alone, patients with NASH are at higher risk of liver-related mortality [12], and for this reason, one of the major clinical challenges today is differentiating patients with steatosis from those with underlying NASH. Since performing liver biopsy on a third of the US population is unreasonable, the availability of accurate, noninvasive markers of fibrosis will be crucial for successful individualized management of disease activity in patients [13•].

The “Ideal” Noninvasive Liver Fibrosis Biomarker

Several noninvasive approaches, including scoring systems that utilize direct serum markers of liver fibrosis based on underlying biology as well as some exciting technological advances in imaging, are under investigation and have been reviewed comprehensively elsewhere [13•, 14, 15•, 16]. It has been proposed that the ideal noninvasive biomarker for liver fibrosis should be (1) readily available, inexpensive, safe, and reproducible; (2) highly sensitive to changes in liver fibrosis stage; (3) useful for monitoring disease progression during the natural history of disease as well disease regression in response to therapy; and (4) free from false positive results related to other disease pathologies

such as inflammation or steatosis [13•]. Here, we highlight some of the more promising noninvasive strategies under development for noninvasive detection of fibrosis with respect to the above criteria and also discuss their potential for future applications in the clinic.

In most of the studies discussed, the performance of the noninvasive technique is evaluated against a scored liver biopsy and the area under the receiver operating curve (AUROC) is calculated to determine the sensitivity and specificity for fibrosis diagnosis. However, since the AUROC of a typical 15-mm core liver biopsy is only 0.82 when compared against whole liver sections [17], it is important to note that many of these techniques could in fact diagnose and stage liver fibrosis better than the comparison biopsy.

Noninvasive Serum Biomarkers

Serum biomarkers eliminate many of the concerns associated with liver biopsy. In general, these tests are not only cost effective and minimally invasive but are also associated with very little sampling error and intra/inter-observer variability [13]. Two broad types of serum biomarkers predominate within the field of liver fibrosis staging, direct, and indirect markers (Table 1). Routine serum-based laboratory tests or “indirect” markers include aminotransferase levels, bilirubin, gamma-glutamyl transpeptidase (GGT), prothrombin time, albumin, and platelet (PLT) counts. These markers have traditionally been used clinically for the detection of cirrhosis in conjunction with clinical exam findings. Although they are altered in liver disease, these markers are not specific to liver injury and hence are considered indirect measures of liver injury and fibrosis. As the pathogenesis of liver fibrosis has become better delineated at the molecular and cellular level, additional biomarkers and prediction algorithms have included more “direct” markers of liver fibrosis. These typically represent soluble or secreted proteins that are produced by hepatic stellate cells, which are the principal cellular driver of hepatic fibrosis, or locally within the fibrotic milieu of the diseased liver. These include ECM proteins (hyaluronic acid), matrix metalloproteases (MMPs), MMP inhibitors [tissue inhibitors of matrix metalloproteases (TIMPs)], as well as fragments of procollagen III. Both indirect and direct markers as well as patient age have been combined in varying fashion to produce a number of scoring algorithms that have been used with fair to moderate accuracy in predicting liver fibrosis stage (Table 2).

A majority of serum biomarkers initially developed were tested and validated on patient populations with chronic hepatitis C (CHC). A recent meta-analysis of noninvasive serum biomarker use in CHC suggests that a

Table 1 Indirect and direct noninvasive serum-based algorithms for detecting liver fibrosis

Test	Variables	Formula
Indirect		
APRI	AST, platelets	$[\text{AST (U/L)}/\text{upper limit of normal (U/L)}] \times 100/\text{platelets (10}^9/\text{L)}$
FIB-4	Age, AST, ALT, platelets	$\text{Age} \times \text{AST (U/L)}/[\text{platelets (10}^9/\text{L)} \times \text{ALT}^{1/2} \text{ (U/L)}]$
FibroTest ^a	A2-macroglobulin, haptoglobin, apolipoprotein A1, gamma-glutamyl transpeptidase, total bilirubin	$4.467 \times \log_{10} [\alpha_2\text{-macroglobulin(g/L)}] - 1.357 \times \log_{10}[\text{Haptoglobin(g/L)}] + 1.017 \times \log_{10}[\text{GGT(IU/L)}] + 0.0281 \times [\text{age(years)}] + 1.737 \times \log_{10}[\text{bilirubin (}\mu\text{mol/L)}] - 1.184 \times [\text{ApoA1 * (g/L)}] + 0.301 \times \text{sex(female = 0, male = 1)} - 5.54$
FibroSure ^a	FibroTest	–
NAFLD-FS ^b	Age, BMI, IFG/diabetes, AST, ALT, platelet, albumin	$= -1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{IFG/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet (}\times 10^9/\text{l)} - 0.66 \times \text{albumin (g/dl)}$
Direct		
ELF ^a	Hyaluronic acid, procollagen III amino terminal Peptide, TIMP-1	$-7.412 + [\ln(\text{HA}) * 0.681] + [\ln(\text{PIIINP}) * 0.775] + [\ln(\text{TIMP1}) * 0.494] + 10$ (based on Metavir staging)

^a Patented test and requires assay be performed in a validated laboratory

^b Online score calculator is available: <http://www.nafldscore.com/>

majority of panels have good ability to diagnose advanced fibrosis ($F \geq 2$) but cannot distinguish between early stages with any granularity. Furthermore, a large number of patients are often found in the indeterminate range of scoring systems [18, 19].

Most of these serum biomarkers have been subsequently validated in other disease states. As certain biomarker profiles rely heavily on “indirect” markers of liver injury such as aspartate aminotransferase (AST) or total bilirubin, it is important to keep in mind that false positives can occur especially if these tests are inappropriately used in patients with specific co-morbid conditions or conditions in which the marker was not validated. Two examples of this are FibroTest, which could be falsely elevated in someone with biliary obstruction, Gilbert’s or hemolysis, and the AST to PLT ratio index (APRI), which could be falsely positive in chronic hepatitis B (CHB), where there is excess necroinflammatory activity or acute hepatitis. Of note, only two scores, which have not been externally validated, have been proposed to be used specifically for HBV infection [20, 21]. In addition, noninvasive methods that utilize indirect markers such as AST are not very useful in predicting the presence of advanced fibrosis in patients with ALD due to the well-known effect of acute or chronic alcohol use on AST levels. Because of this, FibroTest, which does not rely on AST, was shown in a study of 218 patients with ALD to outperform FIB-4 index or APRI [22]. A systematic review of noninvasive serum biomarkers in ALD confirmed this more broadly and highlighted the significant heterogeneity in studies but overall supports the use of biomarker panels that incorporate direct markers for the prediction of advanced fibrosis or cirrhosis [23]. Below, we discuss a few of the more useful scoring systems.

FibroTest

FibroTest, which uses a score of zero to 1.0, was the first predictive algorithm for fibrosis as a result of CHC and showed that combinations of biochemical markers could be used with high positive predictive value (PPV) and negative predictive value (NPV) to identify patients with clinically significant fibrosis [19]. This test includes α_2 -macroglobulin, haptoglobin, GGT, γ -globulin, total bilirubin, and apolipoprotein A₁ and was designed in a prospective analysis of two groups of patients with CHC undergoing liver biopsy. The accuracy of this test is high, with an AUROC of 0.836–0.870 and a high NPV for excluding Metavir stage $F \geq 2$ of >90 % for scores from zero to 0.20. A high PPV of >90 % was obtained for scores from 0.8 to 1 in predicting the presence of fibrosis stage $F \geq 2$. The authors of the study estimated that the use of FibroTest could reduce the need for biopsy by up to 46 %. For CHB, a meta-analysis of 1,457 patients revealed a mean AUROC for detection of significant fibrosis of 0.80 [24]. One disadvantage of FibroTest is that it is an expensive patented test that requires samples to be sent out for analysis and in addition about 50 % of patients will have an indeterminate score (0.2–0.8). In the US, FibroTest is marketed by LabCorp under the name FibroSure and utilizes the same parameters but adds age, gender, and alanine aminotransferase (ALT).

APRI

APRI was also first designed in patients with CHC [25]. It is calculated as $\text{APRI} = (\text{AST elevation/PLT count}) \times 100$ and provides results in a range from 0.1 to 8.0. This test has an AUROC of 0.80–0.88 in predicting significant

Table 2 Diagnostic performance of indirect and direct serum biomarker algorithms for detecting significant (advanced where indicated) fibrosis in CHC, CHB, and NAFLD

Test	CHC				CHB				NAFLD			
	Sen (%)	Spec (%)	AUROC	PPV/NPV	Sen	Spec	AUROC	PPV/NPV	Sen (%)	Spec (%)	AUROC	PPV/NPV
<i>Indirect</i>												
APRI			0.80–0.88 [25]				0.72 [100]		75	86	0.85 [34] ^{c,f}	
≤0.5	91	47		0.61/0.86	82.4 %	38.5 %		0.54/0.71				0.54/0.93
>1.5	41	95		0.88/0.64	48.5 %	85.9 %		0.75/0.66				
FIB-4			0.85 [28] ^e								0.80 [35] ^{d,f}	
<1.45	74.3	80.1		0.41/0.95	–	–	–	–	74	71		0.43/0.90
>3.25	37.6	98.2		0.82/0.88	–	–	0.91 [101] ^e		33	98		0.80/0.83
<1.0					91.2	72.8		0.76/0.90				
>2.65					38.5	97.9		0.62/0.95				
FibroTest™			0.87 [19]				0.78 [102]				0.81–0.92 [33] ^b	
<0.2	92	46		0.58/0.87	89 %	52 %		0.43/0.92	92	71		0.33/0.98
>0.8	38	97		0.92/0.62	18 %	99 %		0.92/0.75	25	97		0.60/0.89
<i>NAFLD-FS</i>												
≤−1.455	–	–	–	–	–	–	–	–	82	77	0.88 [32]	0.56/0.93
>0.676	–	–	–	–	–	–	–	–	51	98		0.90/0.85
(≥F3–F4)												
<i>Direct</i>												
ELF™			0.77 [29] ^a									
0.063 to 0.564	95	29		0.27/0.95			0.90 [103]					
(Scheuer 3–4)	30	99		0.90/0.83	86 %	86.5 %		0.94/0.71				
8.5 (≥F2)					83.5	77.7		0.79/0.83				
9.4 (≥F3)											0.82 [36] ^e	
≤−0.6746									90	50		0.54/0.88
>0.5734									45	95		0.85/0.72

Range of AUROC signifies test and validation groups

^a Original European Liver Fibrosis score; since modified to be enhanced liver fibrosis score and values shown here represent performance in HCV subgroup in original study^b Uses cutoff of 0.3 and 0.7^c Uses cutoff of 0.98^d Uses cutoff of 1.30 and 2.67^e AUROC is for Metavir ≥ F3^f AUROC is for NASH CRN ≥ F3

fibrosis (Ishak ≥ 3). Values ≤ 0.5 can predict the absence of significant fibrosis with a NPV of 86 %, while values ≥ 1.5 can predict significant fibrosis with PPV of 88 %. For the prediction of cirrhosis, values ≤ 1 predict the absence of cirrhosis with NPV 98 %, while values ≥ 2 predict cirrhosis with PPV of 57 %. While this test performs moderately well in CHC, it does less well in other causes of chronic liver disease, including CHB, ALD, and NAFLD (AUROC of 0.72, 0.59, and 0.73, respectively). In general, APRI performs worse than other serum scoring systems as it only incorporates two indirect markers of fibrogenesis. For example, the use of APRI to predict significant fibrosis ($F \geq 2$) in CHB has been systematically reviewed in a meta-analysis of nine published studies with a total of 1,798 patients [26]. The study concluded that APRI was not reliable in predicting advanced fibrosis or cirrhosis with AUROC of 0.70 and 0.75, respectively.

FIB-4 Index

The FIB-4 index was first described by the authors of the AIDS Pegasis Ribavirin International Coinfection Trial (APRICOT) as an index that could predict fibrosis stage (Ishak 0–3 vs. Ishak 4–6) in patients co-infected with HIV and HCV [27]. This index has high clinical utility as it can be calculated readily at the bedside by using the patient's age and three indirect markers: ALT, AST, and PLT levels. The FIB-4 formula is $((\text{age expressed in years}) \times (\text{AST [U/L]})) / ((\text{PLT [10}^9/\text{L]}) \times (\text{ALT [U/L]}^{1/2}))$. In a retrospective study, the formula was shown in a cohort of 832 patients to accurately predict the presence or absence of advanced fibrosis (Ishak stage 4–6) in patients that lay outside the 1.45–3.25 range. Using a cutoff of <1.45 , the index had a NPV to exclude advanced fibrosis of 90 % with a sensitivity of 70 %. A score >3.25 had a PPV of 65 % and specificity of 97 % in identifying advanced fibrosis or cirrhosis. Compared to liver biopsy, the test had an AUROC of 0.765 for differentiating Ishak 0–3 from Ishak 4–6.

A subsequent study later demonstrated that the FIB-4 index could also be used to discriminate early fibrosis from advanced fibrosis in HCV-mono-infected patients [28]. The study authors showed that FIB-4 could accurately identify patients with severe fibrosis ($F \geq 3$) with an AUROC of 0.85. Using the same cutoff of <1.45 , FIB-4 could exclude severe fibrosis in CHC with a NPV of 95.2 %. In comparison to FibroTest, FIB-4 was concordant in up to 92 % of cases. The advantages of FIB-4 compared to FibroTest are that it does not suffer from false positives due to elevations in total bilirubin seen in Gilbert's, hemolysis, or biliary obstruction, it is inexpensive, and it can be calculated easily.

The FIB-4 index was also validated prospectively in 668 HBV mono-infected and treatment naive patients undergoing liver biopsy. The authors found FIB-4 value to be highly

accurate in the prediction of significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$) with an AUROC of 0.865, 0.91, and 0.93, respectively. Using cutoffs of 1.0–2.65, up to 60 % of patients can be correctly classified as having severe fibrosis, with NPV and PPV of 90 and 95 %. Similarly, using cutoffs of 1.6–3.6, 70 % of patients can be correctly classified as having cirrhosis, and thus biopsies can be avoided in the majority of patients.

European (Enhanced) Liver Fibrosis (ELF) Panel

The enhanced liver fibrosis (ELF) panel is unique compared to FibroTest, APRI, and FIB-4 in that it incorporates only direct markers of fibrosis (Table 1). This model was initially tested in a large cohort of 921 patients with chronic liver disease due to a variety of conditions (49 % with CHC) [29]. By adopting different test thresholds, the algorithm can identify significant fibrosis (Scheuer stages 3 and 4) with sensitivity and specificity of over 90 %. In the CHC subgroup, the test has an AUROC of 0.77 and a score of 0.063 was able to identify significant fibrosis with a sensitivity of 95 % and a NPV of 94.5 %. The specificity at this level, however, is low. Thus, in CHC, ELF is an excellent test for ruling out significant fibrosis. At the other end of the spectrum, a score of 0.564 provides a sensitivity of 30 % but a specificity of 99 % for significant fibrosis with a PPV of 90 % and NPV of 83 %. Compared to CHC, the ELF algorithm appears to perform better in ALD and NAFLD. The European ELF was later simplified by excluding age to form the ELF score [30]. This second generation ELF score was further validated using three cohorts of CHC patients (347 patients in total) and showed a pooled AUROC of 0.85 in detecting severe fibrosis ($F \geq 3$). A value less than 9.59 was able to rule out severe fibrosis with a NPV of 90 % and sensitivity of 85 %, while a value greater than 10.22 could rule in severe fibrosis with a PPV of 68 % and specificity of 85 %.

NAFLD Fibrosis Score

Several noninvasive methods of detecting advanced fibrosis and cirrhosis in patients with NAFLD have been reviewed recently [31•]. The most well studied and validated serum-based model of distinguishing patients with and without advanced fibrosis is the NAFLD fibrosis score [32]. The NAFLD fibrosis score uses six variables and was developed in an initial study including 733 patients for developing ($n = 480$) and validating ($n = 253$) purposes. The test performed well with AUROC of 0.88 and 0.82 in the estimation and validation groups, respectively. In the validation group, it could exclude advanced fibrosis with a NPV of 88 % for patients with score <-1.455 and diagnose advanced fibrosis with a PPV of 82 %. Using these

cutoffs, a biopsy could be avoided in 75 % of patients tested with only a 10 % false prediction rate. FibroTest [33], APRI [34], FIB-4 [35], and ELF [36] have all been studied as well with varying AUROC for detecting advanced fibrosis (0.75–0.86, 0.73–0.85, 0.8–0.86, and 0.90, respectively). Overall, noninvasive tests are excellent for excluding advanced fibrosis with NPV > 90 %, but are less accurate for excluding or detecting milder forms of fibrosis.

CD163—A Novel Direct Serum Marker

Kupffer cells are resident macrophages in the liver and express the cell surface marker CD163. The extracellular ectodomain of CD163 is shed into the circulation upon activation and levels of cell surface and soluble CD163 (sCD163) have been shown to correlate with liver injury in acute viral hepatitis as well as liver dysfunction and portal hypertension in patients with cirrhosis [37–40]. A recent study generated a fibrosis score unique to CHC utilizing sCD163 (CD163-HCV-FS) [41••]. When compared to FIB-4 and APRI, CD163-HCV-FS outperformed both in predicting significant fibrosis (Scheuer $\geq$ grade 2) with AUROC of 0.79 versus 0.74 and 0.75, respectively. A cutoff of 1.55 could rule out significant fibrosis in CHC with a NPV of 82 % and sensitivity of 90 %. CD163-HCV-FS $\geq$ 3.5 could rule in significant fibrosis with PPV of 82 % and specificity of 93 %. Further, CD163-HCV-FS outperformed APRI in predicting advanced fibrosis or cirrhosis and was equivalent to FIB-4 (AUROC 0.86 vs. 0.81 and 0.90 vs. 0.85). Although this scoring system is unique in that it utilizes a marker that directly reflects disease activity, it has modest predictive power based on NPV and PPV and requires further validation.

Noninvasive Imaging Techniques

Unfortunately, conventional imaging is not able to detect fibrosis at a mild or moderate stage [42]. However, advanced imaging techniques involving elastography to measure liver stiffness have been developed over the last decade and are very accurate for detecting liver fibrosis (Table 3). In addition, several probes that target ECM proteins have been evaluated in animal models of liver fibrosis in the past several years, and these molecular imaging strategies hold great promise not only for monitoring disease progression but also in response to antifibrotic therapies.

Transient Elastography (TE)

TE (Fibroscan) [43] is the most studied noninvasive technique used to image liver fibrosis. During the procedure, a

low frequency vibration is transmitted through the right lobe of the liver using an ultrasonic transducer on the skin, and the velocity of the returning shear wave is converted into a liver stiffness measurement (LSM) using Hook's law [9]. TE has shown promise for noninvasive staging of fibrosis but it still only measures roughly 1/500 of the liver volume [42]. A meta-analysis of 50 TE studies of the liver concluded that the technique can be performed with excellent diagnostic accuracy [44]. The AUROC for detecting significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$) was 0.84, 0.89, and 0.94, respectively. While the diagnosis of cirrhosis was independent of the underlying liver disease, there was a high variation of the AUROC for the diagnosis of significant fibrosis, as it was dependent on the underlying liver disease. In fact, TE test performance is low in patients with ascites or morbid obesity [45]. In ALD, TE can accurately assess severe fibrosis ($F \geq 3$) and cirrhosis ($F = 4$) with AUROC of 0.94 and 0.87, respectively. However, LSM has been shown to decrease in ALD patients after just a few days of abstinence, this caveat should be taken into consideration when analyzing results [46]. A meta-analysis of 18 studies of HBV patients revealed similar results to HCV patients with AUROC of 0.859, 0.887, and 0.929 for significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$), respectively [47].

Acoustic Radiation Force Impulse (ARFI)

LSM can also be assessed by ARFI during a conventional ultrasound, allowing the exact localization of the measurement site. Because of this, a meta-analysis of 13 studies determined that ARFI had a similar predictive value as TE for significant fibrosis ($F \geq 2$) and cirrhosis ($F = 4$) but could be performed with a higher rate of reliable measurements [48••]. Another meta-analysis study of 8 studies with 518 patients determined AUROC of 0.87, 0.91, and 0.93 for significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$), respectively [49]. Results with ARFI are not confounded by steatosis. In fact, ARFI was shown to distinguish simple steatosis from NASH with an AUROC of 0.867 in 64 NAFLD patients. In addition, the AUROC for detecting significant fibrosis ($F \geq 2$) and cirrhosis ($F = 4$) in NAFLD was 0.944 and 0.984, respectively [50]. However, some reports have reported no significant differences between ARFI and TE for the diagnosis of fibrosis in NAFLD [51]. ARFI also demonstrated good diagnostic accuracy in 112 ALD patients with AUROC of 0.846, 0.875, and 0.893 for significant fibrosis (Scheuer ≥ 2), severe fibrosis (Scheuer ≥ 3), and cirrhosis (Scheuer = 4) [52]. Importantly, ARFI is less expensive than TE as it only requires some additional software integrated into traditional ultrasound equipment.

Table 3 Diagnostic performance of imaging modalities for detecting significant fibrosis ($F \geq 2$) in patients with chronic liver disease of varying etiologies

Etiology	Analysis	Cutoff	Sensitivity (%)	Specificity (%)	AUROC	PPV	NPV
TE							
Mixed [44]	Meta-analysis	–	–	–	0.840	–	–
HBV [47]	Meta-analysis	7.90 kPa	74.3	78.3	0.859	–	–
ARFI							
Mixed [48••]	Meta-analysis	–	74.0	83.0	0.850	–	–
Mixed [49]	Meta-analysis	1.34 m/s	79.0	85.0	0.870	0.91	0.66
NAFLD [50]	Single center	1.17 m/s	84.8	90.3	0.944	0.90	0.85
ALD [52]	Single-center	1.27 m/s	77.0	85.0	0.846	0.89	0.70
SSI							
HCV [54••]	Single-center	9.12 kPa	72.0	81.0	0.948	–	–
Mixed [55]	Single-center	8.0 kPa	83.0	82.0	0.890	0.88	0.75
MRE							
HBV [58]	Single-center	4.07 kPa	95.0	94.5	0.986	0.96	0.93
Mixed [59••]	Meta-analysis	–	87.0	94.0	0.970	–	–
Mixed [60]	Single-center	2.49 kPa	100.0	91.0	0.994	0.93	100.0

Supersonic Shear Imaging (SSI)

SSI measures resulting shear waves from a radiation force and like ARFI can also be measured during a standard ultrasonographic examination. LSM using SSI is fast, repeatable, and reproducible [53]. A pilot study in 113 CHC patients demonstrated that SSI was more accurate than TE with AUROC of 0.948, 0.962, and 0.968 for significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$) [54••]. Likewise, a more recent study of 349 patients with mixed etiologies demonstrated that SSI had a higher degree of accuracy than ARFI for significant fibrosis ($F \geq 2$) and was more accurate than TE for the diagnosis of severe fibrosis ($F \geq 3$) [55]. However, another study found that a significantly higher number of reliable measurements were achieved with ARFI as compared to SSI and TE as higher body mass index was associated with unreliable measurements for both SSI and TE [56].

Magnetic Resonance Elastography (MRE)

Compared to TE and ARFI, MRE has the advantage of whole liver coverage [57]. In a recent study of 113 CHB patients, MRE showed excellent diagnostic accuracy for fibrosis with AUROC of 0.961, 0.986, 1.000, and 0.998 for mild fibrosis ($F \geq 1$), significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis ($F = 4$) [58]. A recent meta-analysis comparing MRE data from 982 patients across 11 studies to ARFI data from 2,128 patients across 15 studies demonstrated that MRE is more accurate than ARFI especially at earlier stages [59••]. The AUROC for mild fibrosis ($F \geq 1$), significant fibrosis ($F \geq 2$), severe fibrosis

($F \geq 3$), and cirrhosis ($F = 4$) was 0.94, 0.97, 0.96, and 0.97 for MRE and 0.82, 0.85, 0.94, and 0.94 for ARFI. In a single center study, this MRE was also reported to have a higher technical success rate and diagnostic accuracy compared to TE [60], although this needs to be demonstrated in multicenter trials. Compared to TE and ARFI, MRE is the most expensive technique and this may limit widespread implementation.

Magnetic Resonance Imaging (MRI)

MRI offers several technical advantages compared to other imaging techniques including its deep tissue penetration, high spatial resolution, and complete coverage of the entire liver. In addition, MRI does not require ionizing radiation which is beneficial in monitoring a disease that is known to take years to progress or regress and thus would most likely require several rounds of imaging. For these reasons, several studies have examined different MRI techniques for their ability to diagnose fibrosis. In a small study, whole liver T1 ρ MRI could accurately assess the different Child-Pugh classes of cirrhosis [61]. Further studies are warranted to see if this technique can distinguish earlier fibrosis stages. Diffusion-weighted MRI can detect fibrosis, but unfortunately apparent diffusion coefficient (ADC) measurements cannot distinguish stages of disease [62–64].

Molecular MRI

While great strides in molecular imaging have been made for oncology, neurology, and cardiovascular disease [65], efforts in the liver and for organ fibrosis have been lacking

thus far. Liver fibrosis is characterized by excess deposition of ECM proteins and several probes have been developed to molecularly image changes in their expression during liver fibrosis. For example, a gadolinium-labeled peptide that detects fibronectin-fibrin complexes (termed Gd-P) was shown to detect liver fibrosis in carbon tetrachloride (CCl₄)-injured mice [66]. Unfortunately, Gd-P could not distinguish mice injured with CCl₄ for 4 weeks from those injured with CCl₄ for 8 weeks. Likewise, a gadolinium-containing elastin-specific contrast agent (termed ESMA) could detect fibrosis in mice injured with CCl₄ [67]. However, unlike Type I collagen, elastin only accumulates in late stage fibrosis [68], and therefore it is unclear whether this contrast agent will be successful in distinguishing different stages of liver fibrosis.

We hypothesized that an MRI agent providing a measure of collagen levels would have utility in staging liver fibrosis and developed a gadolinium-labeled peptide that binds to Type I collagen (termed EP-3533) [69•]. Type I collagen is an attractive target because its concentration increases as fibrosis progresses [17] and its extracellular location makes it readily accessible to the probe. We observed a strong positive linear correlation between imaging with EP-3533 and liver collagen levels as assessed by hydroxyproline analysis ($r = 0.89$) [70••]. In addition, the AUROC for detecting fibrosis (Ishak 0 vs. Ishak ≥ 2) and for distinguishing early from late fibrosis (Ishak ≤ 3 vs. Ishak ≥ 4) was 0.933 and 0.942, respectively. EP-3533 has a blood half-life of 19 min and is largely eliminated from the body at 24 h with only a small retention in the bone and kidney. In addition, EP-3533 has no measurable effect of inhibiting receptor binding or enzymatic activity in an in vitro lead side-effect panel.

While none of these molecular imaging approaches have been evaluated in humans yet, our results with EP-3533 demonstrate that they should be safe for translation. Importantly, they potentially hold great promise as liver fibrosis is a dynamic process and the expression of these ECM proteins should reflect changes in tissue remodeling during both fibrosis progression and regression.

Future Directions

Most of these techniques have been developed as noninvasive surrogates for liver biopsy in order to diagnose and stage liver fibrosis. However, over the last several years, multiple studies have begun to evaluate these tools in additional settings—most importantly as biomarkers for response to antifibrotic therapies and as predictive models for liver outcomes including hepatic decompensation and HCC development.

Monitoring Response to Antifibrotic Therapies

At its early stages, fibrosis is reversible [71, 72] and causal treatment (cessation of alcohol intake, weight loss and glucose control, viral clearance, or suppression) improves liver function [42]. There are also ongoing efforts to develop agents that block fibrosis progression and/or reverse established fibrosis in instances when the underlying insult cannot be removed or when fibrosis has progressed to a late stage. In fact, there are preclinical and clinical trials of a number of antifibrotic therapies that interrupt several steps in the fibrotic pathway [73–77], but a major obstacle to their development has been the slow progression of the disease in humans, coupled with a lack of sensitive and noninvasive means to assess fibrosis or active fibrogenesis [42]. Together, these factors create an enormous cost risk for antifibrotic drug development, since clinical trials require large patient populations treated for long periods of time to reach a clinically significant endpoint. Thus, a biomarker of fibrosis that could accurately assess fibrogenesis early in treatment would not only be extraordinarily useful in enabling evaluation of much larger pools of candidate therapies in clinical trials but also might provide the early evidence of efficacy needed to incentivize investigators and pharmaceutical sponsors to support long-term trials [78].

To date, most noninvasive strategies have been examined in the setting of antiviral therapy. For example, FibroTest score and LSM have been reported to decrease in patients after receiving antiviral therapy for HCV, regardless of virological response [79••], while another study found that LSM values only continued to decrease after therapy in those patients that achieved a sustained virological response (SVR) [80]. Likewise, FibroTest score [81] and LSM [82] have been shown to decrease in patients receiving antiviral therapy for HBV.

Predicting Liver Outcomes

The liver offers a unique setting for early cancer detection because the patients at highest risk for developing liver cancer are well defined and routinely seen by physicians for their cirrhosis. In fact, the major clinical consequences of cirrhosis are impaired liver function and development of HCC, both of which increase the risk of death [83]. End-stage liver disease, including decompensated cirrhosis and HCC, is a major cause of mortality worldwide. In the US, the incidence of HCC has tripled during the past two decades, while the 5-year survival has remained below 12 % making HCC the most rapidly increasing cause of cancer-related mortality [84]. The majority of patients present with advanced disease and as such, current therapies are ineffective for most HCC patients [85].

Identification of high-risk populations suitable for screening has been proposed as an alternative strategy to reduce the high rate of mortality associated with this disease as early HCCs are more amenable to treatment [86].

Fibrosis and especially cirrhosis are the major predictors of liver-related morbidity and mortality [42], and measurements of the collagen proportional area in liver biopsies have been shown to predict future hepatic decompensation and HCC development [87]. Unfortunately, biopsy is invasive and not suited for screening due to complications [88]. For that reason, noninvasive techniques to predict future clinical outcomes are urgently needed. One study has reported that baseline LSM in CHB patients could predict both future hepatic decompensation and HCC development with AUROC of 0.820 and 0.789, respectively [89]. However, a more recent study in patients with compensated cirrhosis demonstrated that baseline LSM could predict portal hypertension and hepatic decompensation with AUROC values of 0.744 and 0.929 but were not associated with development of HCC [90]. In a recent meta-analysis, LSM was associated with risk of hepatic decompensation, HCC, and death, but there was considerable heterogeneity between the studies in the magnitude of effect [91••].

A recent meta-analysis revealed that compared with FIB-4 and APRI only FibroTest performed as well as liver biopsy in predicting 5-year mortality in CHC patients with AUROC of 0.73, 0.66, 0.88, and 0.86, respectively [92••]. Similar results have also been reported where FibroTest could predict HCV-related cirrhosis decompensation and 5-year survival rate with an accuracy equal to if not better than liver biopsy (AUROC of 0.96 vs. 0.87–0.91) [93]. APRI has been evaluated in predicting survival in HCV and HIV co-infected patients and it appears to perform equally well (AUROC 0.88) as that obtained using Child–Turcotte–Pugh (AUROC 0.91) or Model for End-stage Liver disease (AUROC 0.84) scoring [94]. The NAFLD fibrosis score may also be useful in predicting liver-related outcomes such as mortality and transplantation, but this requires further validation [95, 96••].

Conclusion

There has been an explosion of research into noninvasive biomarkers of liver fibrosis over the past decade. In general, noninvasive imaging techniques outperform serum scoring systems and most biomarkers are more accurate for advanced stage disease. More recent studies have started to evaluate combinations of serum biomarkers and imaging to increase diagnostic accuracy [97]. While several biomarkers, like FibroTest and TE, have already been established for the clinical care of HCV, most of these techniques are still be

evaluating for their efficacy in HBV, ALD, and NAFLD [15, 98]. However, it has been proposed that these tests can be used immediately to serve as essential pre-screening tools to rule out advanced disease in order to reduce the ever growing population seeking liver biopsy [13].

There is no doubt that early detection and prevention will be the most effective and rational approach to substantially impact the prognosis of fibrosis patients rather than starting treatment at advanced stage. However, development of antifibrotic therapies will be challenging due to the requirement for larger and longer clinical trials because of slow disease progression. Noninvasive, serial assessment of fibrosis should not only identify patients at high-risk for disease progression but also assess treatment response and therefore boost statistical power in these trials. In addition, noninvasive fibrosis biomarkers could also significantly improve early HCC detection to identify lesions at a stage where potentially curative radical therapies can be applied [99]. While more studies are needed, it is clear that noninvasive biomarkers of fibrosis will have important clinical applications in the future and will be the key to cost-effective management of fibrosis patients and successful implementation of antifibrotic therapies.

Compliance with Ethics Guidelines

Conflict of Interest Daniel L. Motola declares he has no conflict of interest. Peter Caravan declares a consultancy fee from Biogen Idec, not related to this work; grant money received from Sanofi, which funded some of the work done with the collagen-targeted probe cited in this manuscript; ownership in the IP associated with collagen-targeted MRI probe with Collagen Medical LLC; stock in Factor 1A, LLC, which is not related to this article; and NIH Grants EB009062, CA161221, HL116315, and HL109448 which are not related to this article. Raymond T. Chung declares NIH Grant K240778772 which is not related to this article. Bryan C. Fuchs declares NIH Grant CA140861 which is not related to this article.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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