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Noninvasive Markers of Fibrosis and Inflammation in Nonalcoholic Fatty Liver Disease

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Abstract

The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing worldwide. Nonalcoholic steatohepatitis (NASH) and fibrosis are associated with elevated morbidity and mortality, and a means of differentiating these diseases from simple steatosis (SS) is needed. Liver biopsy in all patients with NAFLD is not feasible, thus necessitating a noninvasive method for discerning the presence of inflammation and fibrosis. Of the various serum markers, cytokeratin-18 seems to best predict NASH, the NAFLD Fibrosis Score is most closely correlated with fibrosis, and transient elastography can be used for diagnosis of cirrhosis, or to exclude cirrhosis, although its utility is limited by obesity.

Keywords

nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); noninvasive markers; inflammation; fibrosis; transient elastography; biomarkers; magnetic resonance imaging (MRI); magnetic resonance elastography; proton-density-fat-fraction

Introduction

NAFLD is the leading cause of chronic liver disease in the United States and the western world[1]. NAFLD is defined as the presence of steatosis in at least five percent of hepatocytes

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Compliance with Ethics Guidelines:

Human and Animal Rights and Informed Consent:

With regard to the authors' research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration, its later amendments or comparable ethics standards. In addition, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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on liver biopsy assessment or on imaging in patients who consume little or no alcohol and have no other cause of hepatic steatosis[2,3]. NAFLD can be broadly classified into two subtypes: nonalcoholic fatty liver (NAFL), which is considered to be the largely non-progressive form of NAFLD, and nonalcoholic steatohepatitis (NASH), which is considered to be the progressive form of NAFLD[4]. NASH is a clinicopathologic entity and is typically characterized by the presence of macrovesicular steatosis usually in zone-3, lobular inflammation, ballooning degeneration with or without peri-sinusoidal fibrosis. The fibrosis progression rate in NASH versus NAFL is 7 years per each stage of fibrosis versus 14 years per each stage of fibrosis[5]. Approximately 20% of patients with NAFLD who progress are classified as rapid progressors and identification of rapid fibrosis progressors is a key unmet need in the field[5].

Recent data suggests that fibrosis is the most important histologic lesion in predicting long term outcomes in NASH[6–8]. Therefore, early and prompt detection of any fibrosis and advanced fibrosis is a clinically meaningful diagnostic goal. NASH patients not only have increased rates of progression to cirrhosis, but are also at higher risk of developing cardiovascular events and extrahepatic malignancies[9–12]. NASH is virtually indistinguishable, histologically, from alcoholic fatty liver disease (ALD), making the medical/social history the key differentiating feature between the two entities.

Various scoring systems, such as the NAFLD Activity Score (NAS) and Fatty Liver Inhibition of Progression (FLIP) algorithm have been developed to grade severity of disease[3,13]. For the most part, both scoring systems were designed to be used to standardize interpretation of histological findings in NAFLD studies, and are not meant to be used as clinical tools. Many studies have demonstrated that prognostic outcomes in patients with NAFLD are closely related to the degree of inflammation and fibrosis present at the time of diagnosis or on initial biopsy. In a systematic review of 187 patients with paired biopsies, the median time to progress to advanced fibrosis in patients with NASH was 4.2 years, compared to 13.4 years for those with NAFL alone[14]. Fibrosis, in particular, has been associated with increased mortality in many studies[6,15], where the absence of periportal fibrosis has a negative predictive value (NPV) for liverrelated mortality of 100%[11,16].

Caveats to Liver Biopsy Assessment

The gold standard for diagnosing NASH and/or fibrosis is a liver biopsy, which is fraught with many pitfalls, related to both diagnosis and safety. An “adequate” liver biopsy, defined as one that is at least 1.5 to 2 cm in length, 1.5–2 mm diameter, and containing at least 6–8 portal triads[17], can be difficult to obtain, especially since the sample tends to be more fragmented as the level of fibrosis increases. In addition, the specimen obtained from even an “adequate” liver biopsy is only representative of 1/50,000 of the liver tissue, meaning that the possibility of sampling error, in either under-calling or overcalling the degree of fibrosis, is very real. In NASH patients, the sampling variability/discordance seems to be higher: in patients undergoing 2 liver biopsies concurrently, but at different sites, there is discordance not only among the various features of NASH (steatosis, inflammation and ballooning), but there was also a discordance of at least 1 stage of difference in fibrosis between the two samples in approximately 40% of subjects[18]. Additionally, it has been well documented that

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once the disease has progressed to cirrhosis, the hallmark features of steatosis and hepatocyte ballooning disappear, meaning that, in the past, such patients were often labeled as having cryptogenic cirrhosis[19]. As well, there is some question as to the reproducibility of staging, with some studies showing the increased inter-observer variability in assessing the presence of ballooning and inflammation[20,21].

The risks to the patient with a liver biopsy are manifold, and include bleeding (ranging from 1 in 500 for less severe bleeding, and up to 1 in 2500 to 1 in 10,000 for severe bleeding requiring medical intervention), pain (up to 84%), and even death (at a rate of 9 in 10,000)[22]. Therefore, there is a need for noninvasive markers of fibrosis and/or inflammation, which could replace traditional biopsy as a way to determine disease severity, and thereby predict prognosis. To date, the key noninvasive methods can be grouped into two categories: those based on serum markers of inflammation/fibrosis, and those based on radiographic measures of both hepatic steatosis and of liver stiffness.

Serum Markers

Biomarkers of NecroInflammation and Fibrosis—There are various serum markers that have been shown to correlate with inflammation. Of these, the one most closely associated with the presence of NASH is fragmented cytokeratin-18 (CK-18), an intermediate filament protein found in the liver. Obesity-associated liver injury leads to apoptosis, resulting in the release of caspase-cleaved fragmented CK-18 (M30) into the blood. Various studies, in both the pediatric and adult population, have shown that elevated M30 levels may be able to differentiate between SS and NASH, with an area under the receiver operating curve (AUROC) ranging between 0.71 and 0.93[23,24]; levels correlate positively with fibrosis, the overall NAS and the individual components of NAS (steatosis, ballooning, and lobular inflammation). However, the sensitivity and specificity of the test at the lower 95% CI range is quite low, averaging around 60%[25]. This, combined with the fact that it is not a readily available test, suggests that CK-18 is not the ideal marker for NASH and should not be used as part of routine management.

Other markers of hepatocyte inflammation are leptin, adiponectin, C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor α(TNF-α). Adipokines are enzymes involved in post-prandial lipid metabolism. Many of these (adiponectin, TNF-α, leptin and resistin) are associated with obesity and obesity-related disease[26], including NASH. Adiponectin is thought to be protective; lower levels of adiponectin are seen in NASH than in controls (5476 vs 11548 ng/mL, P=0.00001). Levels are inversely correlated with necroinflammation and fibrosis (OR 5.0; P = 0.009 and OR 8.0, P= 0.003, respectively)[27,28]. Thus, adiponectin may predict the presence of both inflammation and fibrosis, and therefore is useful in differentiating between simple steatosis and NASH. However, this test is not available for routine testing, thereby limiting its usefulness.

Biomarkers of hepatocyte fibrosis that have been extensively studied are hyaluronic acid (HA), leptin (LT), laminin (LN), procollagen III N terminal peptide (P3NP), tissue inhibitor of metalloproteinase 1 (TIMP-1), and fibronectin, most of which are seen in the setting of hepatic stellate cell activation. Of these, HA seems to be the most closely associated with fibrosis severity. In one study of patients with biopsy proven NASH with varying degrees of

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fibrosis[29], HA and LN levels were significantly higher in patients with fibrosis than in those without (HA: 332.65ng/mL vs 55.07ng/mL, P<0.001; LN: 482.26ng/mL vs 270.07ng/mL, P<0.0001). Levels of HA were found to be proportional to the degree of fibrosis. A cut-off of 148.50ng/mL had a sensitivity of 95.7% and a specificity of 96.3% for any degree of fibrosis.

The major drawback to these biomarkers is that they are not routinely available for testing in the clinic. However, in the setting of clinical studies, they may be useful in predicting patients at high risk of having inflammation and/or fibrosis. Although there are no current studies that have done so, a panel/model combining M30, adiponectin, and HA may have a predictive index for both inflammation and fibrosis, but requires testing and validation across several diverse populations.

NAFLD Fibrosis Score—The NAFLD Fibrosis Score (NFS) was derived by Angulo et-al in a multicentre study[30] using anthropometric (weight, body mass index, waist circumference) and laboratory data from 733 patients with biopsy documented NAFLD. The homeostatic model assessment (HOMA) was used to predict insulin resistance, and the presence of the various components of metabolic syndrome was recorded. Of the total cohort, approximately one-third did not have any fibrosis, as determined by liver biopsy. After conducting a multivariate analysis on the remaining 480 patients, only 6 variables were significant in predicting fibrosis: BMI, AST:ALT ratio, platelet count, albumin and hyperglycemia/presence of diabetes. The formula derived using these factors was able to differentiate between advanced fibrosis (F3–4) vs minimal fibrosis (F0–2), with an AUROC of 0.88 ± 0.02. Using the equation and the AUROC, they were able to determine cutoff points to either exclude or predict advanced fibrosis (less than -1.455 and greater than 0.676, respectively). The calculation could be used to predict advanced fibrosis in 77% (273/355) and to exclude significant fibrosis in 92.5% (273/295). In the same study, Angulo et-al cross-validated the model in a separate group (n=253). The utility of the NFS in excluding advanced fibrosis was validated in a separate study comparing multiple noninvasive models for fibrosis[31].

Recent information[32,33] showed that an elevated NFS may also correlate with an increased risk of systemic complications: two separate, retrospective studies comparing NAFLD patients stratified by elevated NFS scores to patients with low scores demonstrated that patients with elevated NFS scores have higher rates of cardiac disease, both at baseline and at follow-up. Takahashi's group also calculated the FIB-4 for patients, and found that this was also seen in patients with FIB-4 elevation at baseline, thereby suggesting that the increased is not due to the presence of diabetes (as the NFS is affected by diabetes, but the FIB-4 is not). This suggests that physicians should have a lower threshold for cardiac investigation in patients with an elevated NFS or FIB-4.

Unfortunately, this still means that any patients with a NFS between −1.455 and 0.676 have an indeterminate stage of fibrosis, thereby requiring a biopsy for staging. In addition, although NAFLD mortality is certainly positively correlated with fibrosis stage, as mentioned previously, any amount of fibrosis is a predictor of worse outcomes. This model groups F1 and F2 patients together with F0 patients, which can give a false sense of security

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regarding disease progression. Thus, the NFS, while excellent at identifying advanced disease, it is often too late at this stage to implement possible treatment options. Further studies to differentiate between true steatosis and NASH/any degree of fibrosis are needed.

FibroTest®/FibroSure—FibroTest®, a panel of serum biomarkers (α2-microglobulin, haptoglobin, apolipoprotein A1, total bilirubin and γ-glutamyltranspeptidase(GGT)) has been validated as a noninvasive marker of fibrosis in various chronic liver diseases, with a high negative predictive value for advanced fibrosis[34–37]. Ratziu et-al[38] examined the utility of the test in predicting advanced fibrosis (F2–4) in NAFLD, comparing two groups with NAFLD (n= 170 in group 1, n=97 in group 2) against a control population of blood donors (n=954). Fibrosis was diagnosed by biopsy in the two NAFLD groups. Fibrotest had an AUROC of 0.86 for predicting F2–4, and 0.92 for detecting F3–4, but had no diagnostic value in predicting NASH. Interestingly, the study also demonstrated that elevated ALT values did not correlate with fibrosis stage.

Although the FibroTest would certainly be useful in potentially identifying patients with fibrosis, it would be difficult to perform this test in the clinic, as some of the tests (α2-microglobulin, haptoglobin, apolipoprotein A1) are not available in most routine lab assays.

Fatty Liver Index (FLI)—The FLI, an algorithm based on BMI, waist circumference, triglycerides and GGT, was developed by Bedogni et-al[39] to predict the presence of NAFLD. Citizens of a small town in Modena, Italy who had participated in the Dionysus Nutrition and Liver Study, who consented to participate in this study, had the requisite labwork, and had suspected liver disease (n=497) were identified as possible subjects. Once other causes of liver disease were excluded, patients with NAFLD (n=216) were compared to patients from the previous study without any known liver disease (n=280). Both laboratory and anthropometric (BMI, skinfold thickness) data were collected on both groups of patients, and a regression model was derived from this data for use in predicting the presence of NAFLD.

The two factors most strongly associated with NAFLD were found to be BMI and waist circumference. Interestingly, GGT was an independent predictor of NAFLD, whereas ALT and AST were not associated with the disease. Similarly, elevated triglycerides (TG), but not insulin, was associated with NAFLD. An FLI<30 ruled out steatosis, whereas an FLI >60 ruled in steatosis (confirmed by sonography).

The key limitation of the FLI, other than that it is derived from a homogeneous population, is that it is used to predict steatosis, not NASH or even fibrosis. Given that it is the presence of inflammation and fibrosis that adversely affects morbidity and mortality, the role of the FLI in risk stratifying patients with NAFLD is limited.

FIB-4—Initially developed to predict advanced fibrosis in patients co-infected with HCV and human immunodeficiency virus (HIV), the FIB-4 index is based on age, AST, ALT and platelet levels, and has been validated in patients with HCV mono-infection. Shah et-al[40] examined the role of FIB-4 in evaluating advanced fibrosis (F3–4) in NASH patients already enrolled in the NASH Clinical Research Network (NASH-CRN) database, using labs done

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closest to the time of biopsy. Of the 541 subjects initially included in the analysis, 317 had NASH. In total, 101 patients had advanced fibrosis, for who the median FIB-4 score was 1.11. The authors also compared the FIB-4 to other noninvasive fibrosis panels in the overall NAFLD population – they found that FIB-4 had the greatest AUROC at 0.802, and that there was a statistically significant difference between the AUROC of FIB-4 and the AUROC of all other noninvasive panels ($P<0.015$) except the NFS, where it approached significance ($P=0.092$). The utility of the FIB-4 in differentiating between F0–1 and higher stages of fibrosis was also tested, and found to have an overall AUROC of 0.75. Similar findings were echoed in other studies[41,42].

Given that the FIB-4 is based on readily available lab parameters and can distinguish between minimal and advanced fibrosis, this seems to be an easy method to rule out fibrosis. However, it cannot be used to rule differentiate between NFL and NASH.

AST to platelet ratio (APRI)—APRI has been demonstrated to correlate well with fibrosis in many chronic liver diseases (CLD)[43,44]. The data as to its role in assessing fibrosis in NAFLD is less clear – some studies suggest that the APRI is useful (AUROC of 0.85, sensitivity of 75–89% and specificity of 75–86%%) in diagnosing advanced fibrosis[45], while many other studies comparing the validity of various fibrosis biomarkers in NAFLD suggest that APRI is less useful, with AUROCs ranging from 0.67 to 0.73[31,42,46]. Therefore, it is likely best used to rule out advanced fibrosis.

Enhanced Liver Fibrosis (ELF) Panel—The biomarkers used in the ELF panel are associated with matrix turnover and therefore are markers of fibrosis: HA, P3NP, and TIMP-1. The panel has been validated in other forms of CLD[47]. When studied in the NASH population, it performed well, with an AUROC of 0.90 for advanced fibrosis (F3,4), or 0.82 to diagnose F2–4. Applying a threshold of −0.2070 to rule out any fibrosis resulted in an AUROC of 0.76, a sensitivity of 61% and a specificity of 80%[48]. Unfortunately, as with the FibroTest, it is based on markers that are not routinely available, thus limiting its clinical use when managing patients.

BARD—The BARD score is a weighted calculation based on BMI, AST, ALT, and the presence of diabetes. Several studies have examined the performance of this scoring system in predicting F3–4 disease. In some studies, BARD did well, with an AUROC of 0.865 (95% CI 0.793–0.920), sensitivity of 88.89% and specificity of 88.89% for a score of 2[49]. The authors found that determinants of advanced fibrosis were obesity, diabetes mellitus, advanced age, a platelet count less than 200×10^3/L, and an AST/ALT ratio >0.8. However, it did not perform as well in other studies, where the AUROC was 0.67, with a sensitivity and specificity of 51.2% and 77.4%, respectively[50]. Its utility seems highest in predicting severe fibrosis, or even cirrhosis, and portal hypertension[51]. Demir et-al[52] found that using the FIB-4 sequentially with the BARD decreased the incidence of misdiagnosis of fibrosis stage. Thus, the role of BARD may be to rule out severe fibrosis, or in conjunction with another noninvasive fibrosis model to predict less severe fibrosis. Table 1 compares the performance of the various noninvasive models of fibrosis.

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Radiographic Measures of Steatosis

Ultrasound is the most common method of diagnosing hepatic steatosis, where a fatty liver will appear "bright" (due to hepatic lipid deposition resulting in increased acoustic interfaces) in comparison to the spleen or renal cortex. Unfortunately, this is seen only when >30% of the hepatic parenchyma is infiltrated by fat[53]; the sensitivity of ultrasound for steatosis ranges from 60–94%, with increased sensitivity as the degree of steatosis increases. Paradoxically, as obesity and BMI increase, scan quality and sensitivity for NAFLD decrease, especially when the BMI is >40kg/m^2. Ultrasound has limited utility in diagnosing NASH or fibrosis. However, given its accessibility and lack of side effects, it often serves as a good primary screening tool for diagnosing NAFLD.

Computed tomography (CT) has been extensively studied for the diagnosis of NAFLD, as it allows for visualization of the entire liver, and can be used to diagnose both diffuse and focal fatty infiltration[54,55]. In a non-enhanced CT, hepatic steatosis can be measured by calculating the attenuation difference between the liver and the spleen (liver:spleen attenuation ratio), where the spleen acts as an internal control. In a fatty liver, the attenuation is decreased in comparison to the spleen. Use of contrast decreases the utility of CT, as timing of the imaging in relation to contrast injection can affect attenuation. Additionally, CT can be used to quantify visceral and subcutaneous adipose tissue (VAT, SAT, respectively); VAT correlates with NASH and adipocytokine release[56]. However, the utility of CT in diagnosing or quantifying steatosis is limited, both due to resource availability and safety (radiation exposure).

Proton magnetic resonance spectroscopy (^{1}H MRS) or MR Imaging (MRI) is an accurate, reproducible quantitative image-based marker of hepatic steatosis, with very low intra-observer variability[57–59]. In the past, liver fat quantification was done by utilization of the Dixon method, where the difference in resonance frequency between water and lipid in various tissues is used to measure fat content[60,61]. However, magnetic resonance imaging proton density fat fraction (MRI-PDFF), a more advanced method, has refined the quantitative steatosis assessment over previous methods and is currently the gold standard for assessment of hepatic steatosis in the setting of clinical trials in NASH[59,62]. MRI-PDFF is also increasingly being used in epidemiologic studies as well for noninvasive liver fat quantification[63]. MRI-PDFF correlates well with histological measures of hepatic steatosis[64–66]. A threshold of 6.4% has a sensitivity of 86% and specificity of 83% to diagnose grade 1 steatosis (<33% of parenchyma is infiltrated by lipid droplets)[67]. MRI-PDFF is better than biopsy in assessing quantitative changes in liver fat in the setting of clinical trials in NASH[62,68].

Controlled Attenuation Parameter—Controlled attenuation parameter (CAP) is a complementary measurement, based on vibration controlled transient elastography (VCTE, or Fibroscan®®). Utilizing the same probe, it can measure the degree of attenuation of the ultrasound wave by hepatic fat, thereby quantifying steatosis, expressed as dB/m. Sasso et-al[69] used CAP to measure steatosis in patients with various forms of CLD, not just NAFLD, and found that the AUROC for detecting >10% and >30% steatosis was 0.91 and 0.95, respectively. In addition, the CAP values were positively correlated with the grade of

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steatosis, with values of <205dB/m consistent with S0, and 321dB/m suggestive of S3. Using a cut-off of 283dB/m yielded a sensitivity of 76%, and a specificity of 79[70]. The accuracy of grading is decreased with adjacent steatosis grades, due to overlapping values. In general, CAP would have lower accuracy than MRI-PDFF but it can be refined in future to improve its performance. It should be noted that until recently, the CAP was only available for the M probe. Studies examining the role of CAP using the XL probe in determining degree of steatosis are still underway.

Novel Ultrasound Methods to Assess Fat—Quantitative Ultrasound (QUS) is a novel sonographic technique to measure various acoustic parameters, one of which is the backscatter coefficient (BSC), which is similar to tissue echogenicity, and can be used to measure hepatic steatosis. In a prospective cohort study, Lin et-al[71] recently examined the role of QUS in diagnosing and quantifying hepatic steatosis, using MRI-PDFF as the gold standard comparator. BSC was found to correlate well with MRI-PDFF measurements of steatosis (Spearman coefficient of 0.8). A BSC cutoff value of 0.0038 has an AUROC of 0.98 (95% CI 0.95–1.00, p<0.0001), sensitivity of 93% and a specificity of 98%.

Although this is a novel technique, and needs further study to assess whether it would be able to predict NASH, given the ease of adaptation of existing tools, this may replace traditional sonography as a method to diagnose NAFLD.

Radiographic Measures of Liver Stiffness as a Surrogate for Fibrosis

Transient Elastography Measurement (TEM)—Fibroscan®® (Echosens, Paris, France) is a one-dimensional measure of liver stiffness obtained by measuring the velocity of a low-frequency (50Hz) shear wave emitted from an ultrasound probe into the liver. It is based on the theory that as the liver becomes increasingly stiff, the higher the velocity of the shear wave. Whereas a liver biopsy only measures 1/50,000,000 of the liver volume, this technique measures a cylindrical volume 1cm wide and 4cm long, approximately 2–7cm below the skin surface[72]. This can be performed at the bedside, by a trained operator, and has been validated as a measure of fibrosis in other CLD, especially viral hepatitis[73]. The transient elastography measurement (TEM) value for a healthy liver is <5.5kPa, and in CLD, readings can range from 7–75kPa, with a higher value correlating with increased stiffness. TEM has been shown to be useful in ruling out any fibrosis, and in diagnosing cirrhosis – in both instances, the readings are at either end of the spectrum, and the negative predictive value in ruling out cirrhosis is 90%[74]. However, it has been shown to be of limited use in F2–3 disease.

A number of studies have examined the role of TEM in NAFLD; the diagnostic accuracy of TEM in NAFLD is good[75,76], with an AUROC of 0.927 for ≥F1. TEM readings are limited by obesity, where a BMI>30kg/m^2 is an independent predictor of failure[77], as well as by the degree of steatosis and inflammation within the hepatocyte. As an attempt to deal with this, a new probe (termed the XL probe) was developed for use in patients with elevated BMIs – measurements made with the XL probe were significantly lower than those made with the M probe (1.7 ± 2.3kPa lower, 95% CI 4–2.1 kPa, P<0.001)[78,79]. However, both the M and XL probes had similar AUROC for ≥F2 fibrosis (0.86 and 0.83, respectively), when assessed in

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obese patients and compared to histological fibrosis scores. Failure rates with the XL probe were lower, at 2% (compared to 10% seen with the M probe, P = 0.002)[80]. Based on these studies, new diagnostic cut-off values were proposed for the XL probe, where a value of 7.0kPa was diagnostic of F2 or greater (compared to 6.2kPa for the M probe). Although this deals with the issue of overweight or obese patients, patients who are morbidly obese (BMI>35), or who have significant inflammation or hepatic steatosis may still have falsely elevated elastography readings.

The Food and Drug Administration (FDA) only recently approved (2013) the use of Fibroscan®® for use in the US, although it has been used in Europe and Canada for a number of years. Although the initial expense is costly, limiting its use in the offices of primary care providers, in the hepatology clinic it is of great use in ruling out fibrosis and in diagnosing advanced fibrosis (F4). However, its use is limited when BM is I>35, which would be seen in a large proportion of NAFLD patients.

Acoustic Radiation Force Impulse (ARFI) Imaging—ARFI is a form of elastography performed on modified commercially available ultrasound machines, an advantage over Fibroscan®®. Short duration acoustic pulses, lasting 262 μs, are used to excite tissue and generate micrometer-scale displacements in tissue. The shear wave velocity is then measured to generate readings of liver (and spleen) stiffness. Readings obtained by ARFI were found to correlate closely with histological fibrosis staging, with AUROCs for predicting ≥F2 and F4 of 0.74 (95% CI 0.64–0.86) and 0.79 (95% CI 0.67–0.91), respectively[81].

The role of ARFI in NAFLD is still being assessed. However, of the studies that were conducted in this population, ARFI was found to have similar results to TEM, but it was not affected by morbid obesity (BMI>40), unlike Fibroscan®®. In a study of 172 patients with NAFLD, a cut-off value of 4.24kPa was found to be able to differentiate advanced fibrosis (F3,4) from less severe disease (F0–2) with a sensitivity of 90% and a specificity of 90%[82]. In a smaller study involving 57 patients with NAFLD, ARFI was compared to Fibroscan®® using both the M and XL probes[83]. Although there was a lower failure rate when using the XL probe than with the M probe, the diagnostic accuracy for advanced fibrosis (≥F2) and cirrhosis was similar between the probes. ARFI was performed in both lobes of the liver, with the result that the left lobe had a higher AUROC for F4 than the right lobe, possibly reflecting the heterogeneic nature of hepatic fibrosis. The success rate for ARFI in the right lobe was 94%, compared to 89% in the left lobe, both of which were higher than the success rate with the M probe, but not the XL probe (80% and 86%, respectively). Given that this can be used with a slight modification of existing ultrasound technology, this may be a tool that can be used widely for detecting advanced fibrosis.

Magnetic Resonance Elastrography (MRE)—MRE involves the use of a passive acoustic driver positioned over the liver and connected to an active acoustic driver (positioned outside of the room), which produces vibrations at roughly 60Hz. These are then transmitted through the body via the passive driver, and the resulting shear wave propagation is captured as images using a modified phase-contrast method[84]. Studies examining the role of MRE in NASH show that a cut-off value of 4.15kPa has good diagnostic accuracy in identifying patients with F3–4 disease (AUROC 0.954, sensitivity = 0.85, specificity =

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0.925). Like ARFI, readings are not affected by obesity, and, uniquely, nor are they affected by the presence of ascites. However, this requires specific machinery and software, thereby limiting its widespread use.

In the MOZART trial[59] to assess the efficacy of Ezetemibe in the treatment of NASH, 3-dimensional MRI was used to reconstruct data from MRI-PDFF derived fat droplet localization and liver stiffness measurements obtained from MR elastography, thereby assessing both hepatic steatosis and fibrosis. The results were compared to histological evaluation of both steatosis and fibrosis, as this technique allowed for 3-dimensional mapping of hepatic steatosis distribution throughout the entire liver, and changes in this parameter, both before and after treatment. This technique is advantageous in that it assesses both steatosis and fibrosis throughout the entire liver, unlike the smaller area of the liver that would be sampled in a biopsy, Fibroscan®, or MRS.

Multiscan is another MRI-based method that is emerging as a useful test to assess the severity of liver disease in NAFLD. It utilized the T1 relaxation properties while concurrently quantifying liver fat and iron content[85]. However, further studies are needed to assess its role in the assessment of the treatment of NASH.

Comparative efficacy of various methods

Although there are a number of tests and techniques to measure steatosis, inflammation, and fibrosis, few studies have compared multiple modalities in a prospective manner until recently. In a study of 102 patients with NAFLD, Cui et-al[86] compared the MRE and various serological fibrosis models, using liver biopsy as the gold standard, to assess which method best predicted advanced fibrosis. Of MRE, APRI, BARD, NFS, AST:ALT ratio, Bonacini cirrhosis discriminant score, Lok index, and the NASH CRN model, MRE had the highest AUROC for advanced fibrosis (0.957, 95% CI 0.918–0.996), where a cut-off of 3.64 kPa had a sensitivity of 92.2% and a specificity of 90.4%. Of the fibrosis models, FIB-4 was the next best at predicting advanced fibrosis, with an AUROC 0.861 (95% CI 0.775–0.946). The 2D MRE was able to correctly classify fibrosis in 76% of patients who had an indeterminate FIB-4 score.

Similarly, Imajo et-al[87] compared MRE, MRI-PDFF, Fibroscan® (including CAP measurements), and serological markers of fibrosis in 142 patients with NAFLD and 10 control patients, and found that MRE was better at predicting a fibrosis stage of 3–4 than Fibroscan® (AUROC of 0.91 with 95% CI 0.86–0.96 compared to 0.82 with 95% CI 0.74–0.89). In keeping with these results, the MRI-PDFF better predicted a steatosis grade>2 than CAP (AUROC 0.90 vs 0.73). This study also examined the role of serological markers of inflammation, such as fragmented CK-18 and ALT, and found that there was no added benefit to measuring these values in addition to either Fibroscan® or MRE.

Conclusions

Many advances have been made in the past few decades regarding the pathophysiology and natural history of NAFLD; perhaps the most important of these is the realization that the presence of hepatocyte necroinflammation or fibrosis has a markedly worse prognosis than

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simple steatosis alone. Although liver biopsy remains the gold standard in assessing these two findings, it is not an ideal test due to sampling error and safety issues. Although there are a number of serum biomarkers of inflammation and fibrosis, as well as predictive models of fibrosis based on these markers, not all of these are readily available for routine prognostication. Scoring systems, based on routine labwork, such as NFS, APRI, and FIB-4, can be easily calculated at the bedside, and can be used to identify patients with advanced fibrosis. Radiographic measures of liver stiffness, such as Fibroscan®, ARFI, and MRE, although they have been shown to correlate well with fibrosis stage, still warrant further study in the setting of NAFLD before they can be used in isolation to guide therapy. MRS-PDFF and MRI-PDFF are accurate, reproducible, and robust quantitative imaging based biomarker for the diagnosis as well as quantification of liver fat content. Both MRS-PDFF and MRI-PDFF are better than liver biopsy in assessing quantitative changes in hepatic steatosis in longitudinal studies, and the MOZART trial demonstrated the feasibility of 2D and 3D MRE and co-localization in NASH and anti-fibrotic trials. MRE is probably the best noninvasive, quantitative, accurate and precise biomarker for noninvasive fibrosis assessment. Ultimately, the role of noninvasive markers of NASH may be to guide selection of patients who require a liver biopsy to stratify disease severity as well as to differentiate who needs to be treated.

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Table 1

Summary of serum biomarkers for NAFLD

Marker	Components	Formula	AUC	Low/High Cut-off Values for Advanced Fibrosis
NAFLD Fibrosis Score	Age, BMI, diabetes, AST, ALT, platelet count, albumin	−1.675 + 0.037×age(yrs) + 0.094 × BMI (kg/m^2) + 1.13×IFG or DM (yes = 1; no = 0) + 0.99 × AST/ALT ratio − 0.013 × plt ($\times 10^9/L$) − 0.66 × albumin (g/dL)	0.81–0.88 (95% CI 0.76–0.88)	< −1.455 to exclude fibrosis; >0.676 to Dx advanced fibrosis
Fatty Liver Index	BMI, waist circumference, triglycerides, GGT	Regression algorithm	0.85 (95% CI 0.81–0.87)	FLI <30: rule out NAFLD FLI>60: rule in NAFLD
APRI	AST, platelets	(AST/ ULN)/plt	0.67 – 0.85	>0.98: advanced fibrosis
Fibrotest (aka Fibrosure)	Bilirubin, GGT, α2-microglobulin, apolipoprotein A1, haptoglobin	Online calculator	0.81 (95% CI 0.74–0.86)	>0.30 : advanced Fibrosis (F2 or higher)
FIB-4	Age, platelet count, AST, ALT	Age × AST/plt × √ALT	0.86 (95% CI 0.758–0.847)	>1.11: F3,4
BARD	BMI, AST:ALT Ratio, diabetes	Weighted sum, where BMI>28 = 1 point, AST/ALT ratio>0.8 = 2 points, and diabetes = 1 points	0.67 – 0.865	>2: associated with F3–4 disease
ELF	HA, PIIINP,TIMP-1		0.90 (95% CI 0.84–0.96)	>0.3576 to rule out F3/4

BMI – body mass index; IFG – impaired fasting glucose; DM – diabetes mellitus; AST – aspartate aminotransferase; ALT: alanine aminotransferase; plt – platelets; CI – confidence interval; TG – triglycerides; ELF – enhanced fibrosis panel; HA – hyaluronic acid; PIIINP – procollagen type III amino-terminal peptide; TIMP-1 – tissue inhibitor of metalloproteinase; N/S: not stated