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CAN NASH BE DIAGNOSED, GRADED AND STAGED NON-INVASIVELY?

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Synopsis

Non-alcoholic bland steatosis and nonalcoholic steatohepatitis (NASH) are stages in the spectrum of nonalcoholic fatty liver disease (NAFLD). NASH may progress to end-stage liver disease with liver-related morbidity and mortality occurring almost exclusively in patients with NASH whose disease had progressed to advanced fibrosis and cirrhosis. Liver biopsy is the only accurate tool available to distinguish between patients with NASH and no NASH and to stage fibrosis which is important for patient counseling and monitoring. Markers of hepatocyte apoptosis such as cytokeratine (CK)-18 measured in plasma hold promise as a non-invasive test for NASH diagnosis. Several scoring systems that combine routine clinical and laboratory variables and some proprietary panels can assist in predicting fibrosis severity. Noninvasive imaging modalities such as ultrasound-based elastography (FibroScan), and particularly magnetic resonance-based elastography (MRE) are reasonably accurate available tools to determine severity of fibrosis in NAFLD, but none of them yet can replace liver biopsy.

Keywords

NAFLD; NASH; noninvasive; CK-18; fibrosis; fibrogenesis; FibroScan; magnetic resonance elastography

Background

Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of fat (mainly triglycerides) in hepatocytes that results from insulin resistance [1]. It is the most common chronic liver disease in the Western world. Data from the National Health and Nutrition Survey from 1988 to 2008 show that the prevalence of common chronic liver diseases have remained stable, except for NAFLD as defined by idiopathic elevation of liver enzymes which is increasing [2]. The clinicopathologic spectrum of NAFLD ranges from bland hepatic steatosis which is clinically associated with a similar long-term prognosis as compared to the general population, to nonalcoholic steatohepatitis (NASH) which when associated with increased liver fibrosis, may progress to cirrhosis and liver failure. As such,

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distinguishing between steatosis and NASH with and without fibrosis has important implications for management and patient counseling.

NAFLD is a growing medical problem affecting any age range, with a reported prevalence of 9.6% among adolescents and preadolescents [3], and 34% among patients aged 30 to 65 years [4]. However, the reported prevalence of this condition varies based on the study population studied and the diagnostic modality used. For instance, liver biopsies performed in otherwise healthy potential liver donors revealed a prevalence of NAFLD (as defined by greater than 30% of steatosis) of 20% [5], whereas studies using MR spectroscopy reported a prevalence of 34% in the general adult population in Dallas County, Texas [4]. Studies using idiopathic elevations in liver enzymes as case definition yielded a wide NAFLD prevalence of 8% to 75% [6–8].

Role of liver biopsy in diagnosing and staging NASH

The decision regarding whom and when to biopsy should take into account whether the information likely to be obtained would affect the patient's care. There are two general indications for performing a liver biopsy in patients with suspected NAFLD:

1. confirming the diagnosis and staging the disease. Establishing the diagnosis, activity grade (degree of inflammation and cellular injury) and stage of fibrosis of NAFLD requires a liver biopsy. Given the high prevalence in the population, the invasive nature of liver biopsy, and the paucity of effective therapies, however, there is often an understandable reluctance to perform liver biopsy for the sole purpose of confirming the diagnosis. In most patients, therefore, the diagnosis of suspected NAFLD is based on clinical and laboratory data, and imaging studies with appropriate exclusion of other liver conditions.

2. determining prognosis based on the severity of liver injury and fibrosis. Liver biopsy is the only investigation that can reliably distinguish between simple steatosis and NASH, as well as stage the extent of fibrosis. The prognosis of NAFLD depends on the severity of liver injury and fibrosis. While most studies suggest that there is no increased mortality associated with simple steatosis, mortality in patients with NASH, particularly those with advanced fibrosis and cirrhosis, is increased as compared to the general population of same age and gender. For instance, the prevalence of cirrhosis and liver related mortality within the first 15 years of diagnosis is less than 1% (0.7% and 0.9% respectively) in patients with bland steatosis, but increases to 11% and 7%, respectively, in patients with NASH [9]. The highest liver related morbidity and mortality is undoubtedly among those patients with advanced (stage 3 or 4) fibrosis [10–12]. Additionally, the identification of early cirrhosis or advanced (bridging) fibrosis may alter management as such patients should undergo upper endoscopies to screen for gastroesophageal varices and periodic liver ultrasound imaging to screen for hepatocellular carcinoma [12]. Hence, in the absence of clinical and radiologic features of cirrhosis, liver biopsy remains the only way to reliably assess prognosis.

Over the last decade, several simple laboratory tests (in isolation or in combination), serum markers of fibrogenesis, and imaging studies (ultrasound, computed tomography and magnetic resonance imaging) have been evaluated as a substitute for liver biopsy in NAFLD and had showed varying degrees of accuracy when compared to liver biopsy. There remains a high degree of interest in accurately diagnosing, grading and staging this disease non-invasively.

Limitations of liver biopsy

The potential drawbacks of liver biopsy are well documented. These include sampling error, problems with inadequate biopsy size, variability in pathologist interpretation, cost and associated morbidity. Liver biopsy samples only a tiny portion, roughly 1/50,000th, of the liver. Sampling error is therefore common, with 30–40% of patients with NAFLD undergoing simultaneous paired liver biopsies having samples differing by at least one fibrosis stage [13,14]. Larger biopsy samples are more likely to demonstrate features supporting a diagnosis of NASH with or without fibrosis, so that small samples are more likely to be associated with diagnostic and fibrosis staging error [13,15]. Inter-observer variability between pathologists is reasonable though imperfect adding to the inaccuracy of liver biopsy for staging purposes [16,17]. Finally, percutaneous liver biopsy is associated with serious complications in 0.3% of cases and a mortality rate of 0.01% [18]. These drawbacks of liver biopsy have led investigators to examine noninvasive markers as potential substitutes in the diagnosis and staging of NASH. The ideal noninvasive test should be simple, reproducible, readily available, less expensive than liver biopsy, able to predict the full spectrum of liver fibrosis stages and to reflect changes occurring with therapy.

Noninvasive diagnosis of steatosis

Clinical and laboratory variables

Three indices have been developed to make the diagnosis of steatosis, one is the steatotest™ a proprietary formula based on the six variables of FibroTest-ActiTest plus body mass index (BMI), cholesterol, triglycerides, and glucose adjusted by age and gender [19]. A cutoff of 0.3 has a sensitivity of 85% or more to make the diagnosis of fatty liver, and a cutoff of 0.7 has a specificity of 80% [19]. The fatty liver index or FLI includes four variables: BMI, waist circumference, triglycerides and GGT [20]. The FLI can be calculated using a specific formula with a score of 30 or less having a sensitivity of 87%, and a score of 60 or more having a specificity of 86% in the diagnosis of steatosis [20]. The lipid accumulation product or LAP includes three variables, waist circumference, triglycerides and gender [21]. These indices [19–21] however did not gain much popularity and they may not add much to the information provided by clinical, laboratory and imaging studies done routinely in patients with suspected NAFLD.

Routine imaging studies

Ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) can noninvasively diagnose fatty infiltration of the liver. Hepatic steatosis causes increased echogenicity on ultrasound, which can be contrasted against the lower echogenicity of the spleen or renal cortex. A similar pattern can be seen with diffuse fibrosis, giving rise to the term “fatty-fibrotic pattern,” although the echo shadows tend to be coarser in the presence of pure fibrosis. The sensitivity and specificity of ultrasound for detecting hepatic steatosis vary from 60% to 94% and 88% to 95%, respectively. However, the sensitivity of ultrasound decreases with lower degrees of fatty infiltration. In the presence of ≥30% fatty infiltration, the sensitivity of ultrasound is 80% compared with a sensitivity of 55% when hepatic fat content is 10% to 19% [22]. In addition, the ultrasonography sensitivity for the detection of steatosis progressively decreases as the BMI increases with a sensitivity as low as 39% in individuals with BMI of 35 kg/m^2 or higher [23].

On non-contrast CT scan images, hepatic steatosis has a low attenuation and appears darker than the spleen. On contrast images, CT scan has a sensitivity between 50% and 86%, and a specificity between 75% and 87% for the detection of steatosis [24,25]. Confounding factors such as iron, copper, or fibrous tissue that alter the Hounsfield density of liver, and

differences in the rate of contrast injection and the timing of the scanning may explain the differences in diagnostic accuracy reported in several studies [24,25]. Overall, the sensitivity of CT at detecting greater than 33% hepatic steatosis is up to 93%, with a positive predictive value of 76% [26]. CT however is not sensitive in detecting mild to moderate amount of steatosis between 5% and 30% [26]

Both MRI and MR spectroscopy are reliable at detecting steatosis and offer good correlation with hepatic fat volume [27,28]. The sensitivity and specificity of MRI in detecting as low as 5% of liver fat infiltration is 85% and 100% respectively [27]. MR spectroscopy studies of the human liver have been based on the ubiquitous protons hydrogen (1H) and phosphorus (31P). More than 5% of hepatic fat content on MR spectroscopy indicates presence of steatosis [28]. However, the routine application of MR images is limited by cost and lack of availability.

Noninvasive diagnosis of NASH

Clinical and laboratory variables

Different indices have been proposed to make the diagnosis of NASH (Table 1). One is the NashTest™, another proprietary formula that includes twelve variables and has an area under de receiving characteristic (AUROC) curve of 0.79 [29]. The indices described by Palekar [30] and Shimada [31] include a combination of several other variables described in Table 1. The reported accuracy of these indices seems fair but the number of patients included was too small and further validation of these indices is needed.

Markers of apoptosis

Cytokeratin (CK) 18 is to date the serum marker of NASH that has been most validated. CK-18 fragments come from apoptosis of hepatocytes accomplished by the enzyme caspase 3. CK-18 fragments can be investigated in liver tissue using immunostaining, or measured in plasma using monoclonal antibodies. In the original study [32], 39 patients with suspected NAFLD were included, and CK-18 plasma values of 395 U/L had a petty high AUROC curve with high sensitivity and specificity to differentiate between patients with NASH and non-NASH. Subsequently, a validation study was conducted and included 139 patients with liver biopsy confirmed NAFLD [33]. The AUROC curve was estimated to be 0.83, with 95% confidence intervals (CI) 0.75, 0.91. A CK-18 plasma value of about 250 U/L had a sensitivity of 0.75 (95% CI 0.64, 0.83) and a specificity of 0.81 (95% CI 0.61, 0.93). Subsequently, a number of validation studies have been reported in the literature essentially reproducing the same results [34]. These studies suggest plasma CK-18 levels may help in distinguishing between simple steatosis from NASH, but the test is far from perfect as indicated by the lower 95% CI in the 0.60 range for sensitivity and specificity. CK-18 assay is commercially available, but still has not been cleared by the FDA

Routine imaging studies

Ultrasonography, CT, and MRI are insensitive in differentiating hepatic steatosis from NASH, and they cannot be used to stage fibrosis [26]. A small CT study of patients with NAFLD found that patients with NASH had increased liver size and increased caudate lobe-to-right lobe size ratio, compared with those with steatosis only [35]. The caudate-to-right lobe size ratio was statistically higher in NASH (mean, 0.43; range, 0.31–0.55) compared with steatosis only (mean, 0.36; range, 0.22–0.47). However, measurements showed considerable overlap in both categories, and it is unlikely that these measurements will be useful in individual patients.

Serum markers of liver fibrosis in NASH

Simple laboratory tests as markers of fibrosis

Several routinely available laboratory tests may be abnormal in the presence of advanced liver fibrosis. Markers of synthetic liver function, such as albumin and prothrombin time, often are altered in the presence of cirrhosis, and serum bilirubin may be increased. A low platelet count in the setting of advanced liver disease is usually a sign of hypersplenism related to portal hypertension. Advanced liver disease is often already clinically and radiologically apparent, however, when these laboratory markers become abnormal. Although these markers may assist in assessing the severity of liver decompensation, they are insensitive at detecting non-cirrhotic stages of fibrosis.

Elevated aminotransferase levels are found to correlate with liver fibrosis in certain select populations of patients with NAFLD, such as those undergoing bariatric surgery. Among nearly 1000 morbidly obese subjects undergoing gastrointestinal bariatric surgery in Italy, an aspartate aminotransferase (AST) or ALT level greater than twice the upper limit of normal had a positive predictive value (PPV) for bridging fibrosis of 21% and a negative predictive value (NPV) of 93% [36]. An AST greater than twice the upper limit of normal was also independently predictive of portal or bridging fibrosis in an Asian study of 60 patients with NAFLD [37]. However, other studies have failed to confirm an association between simple aminotransferase levels and degree of fibrosis in patients who have NAFLD [38–40]. Furthermore, studies comparing NAFLD patients who had persistently raised ALT levels to those who had persistently normal ALT levels found no difference in the prevalence of advanced fibrosis and cirrhosis between the groups [41,42]. The association between aminotransferase levels and fibrosis is therefore inconsistent and cannot sufficiently predict fibrosis stage in individual patients.

An association between an elevated AST/ALT ratio and fibrosis has been recognized in chronic liver disease and may reflect impaired AST clearance by sinusoidal cells in the liver [43]. Among patients who have NAFLD without advanced fibrosis, the AST/ALT ratio is typically less than 1, but it tends to reverse as the degree of fibrosis progresses to bridging fibrosis or cirrhosis [38]. Consequently, several studies have found an association between advanced fibrosis on liver biopsy and an AST/ALT ratio greater than 1. In an early study examining 144 biopsy-proven cases of NASH found an AST/ALT ratio greater than 1 remained significantly associated with advanced fibrosis when adjusted for multiple factors [38]. In that study, 82% of patients who had an AST/ALT ratio less than or equal to 1 did not have fibrosis, whereas 47% of those who had a ratio greater than 1 had advanced fibrosis, indicating that the AST/ALT ratio may be a useful clinical adjunct for predicting or excluding advanced fibrosis in patients with NASH.

Another simple laboratory ratio proposed as a marker of advanced fibrosis is the AST-to-platelet ratio index (APRI). While it was initially proposed as a marker of fibrosis in chronic hepatitis C infection, it has been validated in a cohort of 111 patients with NAFLD [44]. In this validation study, the APRI was significantly higher in NASH patients who have advanced fibrosis. The AUROC curve for APRI was 0.85 with an optimal cut-off of 0.98, leading to a sensitivity and specificity of 75% and 86% respectively. The PPV of the APRI was only 54%, with a NPV of 93%. The APRI may therefore be useful in identifying patients unlikely to have advanced fibrosis, but is less useful in predicting the presence of advanced fibrosis.

Serum ferritin levels are elevated in 21% to 40% of patients who have NAFLD and seem related to insulin resistance and liver damage rather than reflecting increased hepatic iron stores [40,45]. Ferritin has been found to be a significant independent predictor of severe

fibrosis in 167 subjects from Italy who had NAFLD [45]. While several studies have failed to replicate this, a large recent study showed a serum ferritin greater than 1.5 times the upper limit of normal was associated with advanced hepatic fibrosis in a cohort of 628 patients with NAFLD [46]. The precise role for isolated serum ferritin as a marker of fibrosis is unsettled. Manousou et al. have recently found that serum ferritin, particularly when combined with an elevated BMI, is a useful discriminant marker for liver fibrosis in patients with NAFLD [47]. This combination may therefore prompt consideration for obtaining a liver biopsy to identify the severity of fibrosis.

Combination of simple laboratory tests and clinical markers of liver fibrosis

In an effort to increase the predictive value of simple laboratory parameters for liver fibrosis, several routine laboratory tests and clinical variables have been identified by multivariate analyses (Table 2) [38, 48–53]. As insulin resistance is a driving force behind the pathogenesis of NAFLD and is associated with stimulating fibrogenic hepatic growth factors [54,55], it is not surprising that the clinical correlates of insulin resistance (obesity, diabetes mellitus, and hypertriglyceridemia) are associated with advanced fibrosis and are incorporated with laboratory tests to predict liver fibrosis.

Among 144 patients who had biopsy-proved NASH, 66% of those who had the combination of obesity, diabetes, age 45 years or older, and AST/ALT ratio greater than 1 had bridging fibrosis or cirrhosis [38]. In contrast, no patient had severe fibrosis in the absence of all of these factors. Another study found age greater than or equal to 50 years, BMI greater than or equal to 28 kg/m2, elevated serum triglyceride, and ALT levels associated with septal fibrosis in a French cohort of 93 obese subjects who had abnormal liver tests [48]. No patient in their cohort who had one or fewer of these factors had septal fibrosis, whereas all four patients who had all four factors had septal fibrosis. Additionally, an Australian algorithm involving systemic hypertension, elevated ALT, and insulin resistance (the HAIR index) provided a sensitivity and specificity of 80% and 89%, respectively, for detecting NASH in patients who were morbidly obese and undergoing bariatric surgery [49]. In the presence of at least two of the three predictive factors in the HAIR index, 10 of 11 patients who had bridging fibrosis or cirrhosis were identified. Unfortunately, the specificity of the index was low, with at least 11 other patients who had a score of 2 or more not having advanced fibrosis.

Based on the results of multivariate analyses, several predictive scores for advanced fibrosis have been developed based on a combination of clinical and routine laboratory parameters. They are the NAFLD fibrosis score, the BARD score, and the FIB-4 index (Table 3) [51,52,56]. These score formulas may assist in deciding when to perform a liver biopsy for fibrosis staging.

In an international multicenter study, data from 733 patients with liver-biopsy confirmed NAFLD was analyzed to create (480 patients) and validate (253 patients) a scoring system to distinguish between patients with (stage 3–4) and without (stage 0–2) advanced fibrosis using Kleiner's staging system [51]. The NAFLD fibrosis score was created using six variables (as shown in Table 3) that were significant by multivariate analysis. The AUROC curve for this score to distinguish between patients with and without advanced fibrosis was high, 0.88 in the estimation group and 0.82 in the validation group. A score less than −1.455 had high accuracy in excluding advanced fibrosis with a NPV of 93% and 88% in the training and validation groups, respectively; whereas a score greater than 0.676 had high accuracy in identifying advanced fibrosis with a PPV of 90 and 92% respectively, in the training and validation groups. If the NAFLD fibrosis score had been applied to the entire cohort of 733 patients, the liver biopsy for fibrosis staging could have been avoided in 75% of patients, that is those correctly identified, and performed in only the 25% of patients that

fell in the indeterminate range. Several studies of independent populations have since reproduced the high accuracy of the NAFLD fibrosis score in distinguishing patients with and without advanced fibrosis [57,58]. In one study of 162 Chinese patients (low prevalence of NAFLD), the lower cutoff value of less than −1.455 was used and had a NPV of 91% in excluding advanced fibrosis [57]. Qureshi et al. evaluated 331 morbidly obese patients with NAFLD who underwent bariatric surgery [58]. The lower cut-off score had a NPV of 98% in ruling out advanced fibrosis.

The BARD score was created by analyzing data collected retrospectively from a group of 827 patients with NAFLD [52]. Based on logistic regression analysis, the BARD score included a combination of three variables as shown in Table 3. They reported a score of 2–4 associated with an odds ratio of 17 for advanced fibrosis. It was unclear how many of the 827 retrospectively evaluated patients had the three variables measured and were in fact included in the evaluation of the BARD score. A BARD score of 2–4 was associated with an odds ratio for advanced fibrosis of 17.3 and a negative predictive value of 97%. The BARD score has since been cross-validated in a Polish population of 104 patients wih NAFLD [59].

The FIB-4 score was originally developed to predict advanced fibrosis in patients co-infected with hepatitis C and HIV. The FIB-4 score was validated in a database of 541 patients with NAFLD to calculate jackknife validated AUROC curves based on 4 variables as shown in table 3 [56]. The AUROC curve was 0.80. Using a cut-off or greater than of equal to 2.67, the PPV was 80% and the NPV was 83%. Using a cut-off of less that 1.30, the PPV was only 43% but the NPV was 90%, suggesting that the FIB-4 index may be useful in excluding patients without advanced fibrosis. The FIB-4 score has been cross-validated in a cohort of 576 Japanese patients with biopsy proven NAFLD [60]. However, the cut-off values used were different from those used in the original study [56]. In the Japanese study [60], the lower cut-off used was less than 1.45. Only 6 of 308 patients with a FIB4 index below the proposed low cut-off point (<1.45) were under-staged, giving a high negative predictive value of 98%. Twenty-eight of 59 patients with a FIB4 index above the high cut-off point (>3.25) were over-staged, giving a low positive predictive value of 53%. Using these cutoffs, 91% of the 395 patients with FIB-4 values outside 1.45–3.25 would be correctly classified, and implementation of the FIB4 index in the Japanese population would be estimated to avoid 58% of liver biopsies.

The performance characteristics of the NAFLD fibrosis score, BARD score, FIB-4 score as well as the AST/ALT ratio and APRI have been compared in an independent population of 145 patients from the United Kingdom [61]. The AUROC curve to distinguish between patients with and without advanced (stage3–4) fibrosis was 0.86 for the FIB-4 score, 0.83 for the AST/ALT ratio, 0.81 for the NAFLD fibrosis score, 0.77 for the BARD score, and 0.67 for the AST to platelet ratio index. The AST/ALT ratio, BARD score, FIB-4 and NAFLD fibrosis scores all had NPVs between 92% and 95% to rule out advanced fibrosis, though positive predictive values were modest for all of them. Based on the data from this study, in order to exclude advanced fibrosis, liver biopsy could potentially be avoided in 69% with AST/ALT ratio, 62% with FIB-4, 52% with NAFLD fibrosis score and 38% with BARD. Adams et al. [62] calculated simple (APRI, BARD) and complex (e.g. hepascore, Fibrotest, FIB4) fibrosis models in 242 NAFLD subjects undergoing liver biopsy. For significant fibrosis (stage 2–4), non-invasive fibrosis models had modest accuracy (AUC 0.707–0.743) with BARD being least accurate (AUC 0.609, $P < 0.05$ vs others). For advanced fibrosis (stage 3–4), complex models were more accurate than BARD (AUC 0.802–0.858 vs 0.701, $P < 0.05$). The authors concluded that in NAFLD subjects, non-invasive models have modest accuracy for determining significant fibrosis and have predictive values less than 90% in the majority of subjects, though complex models are more accurate than simple models across a range of fibrosis.

While these combined clinical and laboratory models may be useful in identifying a subset of patients at low risk of advanced liver fibrosis and who may therefore avoid liver biopsy for staging and prognostic purposes, they are not sufficiently accurate to replace liver biopsy for staging and prognostic purposes if advanced fibrosis is suspected.

Direct serum markers of liver fibrogenesis

Hepatic fibrosis is a dynamic process involving complex interaction between enzymes involved in extracellular matrix synthesis and degradation. Extracellular matrix components, such as hyaluronic acid, collagen components (type IV collagen and type III procollagen peptide, P3NP), and laminin circulate in the serum at low levels and have been examined in isolation and in combination as potential predictors of liver fibrosis in NAFLD (Table 4) [63–70].

Serum levels of hyaluronic acid are increased in liver fibrosis reflecting increased deposition of collagen and decreased clearance by sinusoidal endothelial cells [63]. Several studies have determined that hyaluronic acid predicts bridging fibrosis or cirrhosis in patients who have NAFLD, with accuracy between 80% and 89% [63–65]. Hyaluronic acid, however, is less accurate for detecting lesser degrees of fibrosis with an area under the receiver operator characteristic curve (AUC) for any degree of fibrosis varying between 0.67 and 0.73 [63,66]. In addition, hyaluronic acid increases in systemic inflammatory conditions, which may produce falsely positive predictive results.

Type IV collagen is a product of collagen degradation and a marker of fibrolysis. Serum levels of the 7S domain are increased in the presence of severe fibrosis in patients who have NAFLD. Among 112 Japanese patients who had NAFLD, a cut-off point of 5.0 ng/ml, provided a PPV and NPV of 68% and 84%, respectively, for the presence of severe fibrosis [64]. Laminin is a component of extracellular matrix cleared by hepatic endothelial cells. A small study found levels greater than 282 ng/mL reasonably predictive for the presence of any fibrosis in 30 patients who had NAFLD [66]. Serum YKL-40 is glycoprotein secreted by several different cell types, including hepatic stellate cells. Elevated serum YKL-40 levels have been proposed as a marker of fibrosis in NAFLD [71], based on a small study that lacked a validation group. However, other studies have failed to show an association between YKL-40 and fibrosis in NAFLD [72].

Proprietary predictive panels

To increase the accuracy of noninvasive markers of liver fibrosis, multiple serum markers have been combined into mathematic models to produce predictive scores. The Fibrotest is one algorithm, consisting of a combination of age, gender, bilirubin, g-glutamyltransferase, apolipoprotein A1, haptoglobin, and a2-macroglobulin. It has been validated in a variety of chronic liver conditions and was examined in a cohort of 267 patients, 85% of whom had NAFLD [67]. A score of less than 0.3 (range 0.0 to 1.0) provided a NPV of 98% for the presence of bridging fibrosis or cirrhosis, whereas a score of greater than 0.7 provided a 60% PPV for bridging fibrosis or cirrhosis. However, 33% of individuals had a score between 0.3 and 0.7, indicating that the Fibrotest cannot predict severity of liver fibrosis in one-third of patients with NAFLD.

The European Liver Fibrosis Group assessed the combination of age and serum levels of hyaluronic acid, aminoterminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1 in predicting advanced fibrosis in patients who had a wide range of liver diseases [73]. The proposed algorithm had an acceptable accuracy overall, but only 61 out of the 912 patients studied had NAFLD - a number too small to derive meaningful conclusions about the NAFLD population. The same group therefore evaluated the same three serum

markers: hyaluronic acid, aminoterminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1 (named Enhanced Liver Fibrosis panel, ELF) in predicting fibrosis in 192 patients with NAFLD [68]. An ELF score of 0.3576 had an area under the ROC curve of 0.93, and a sensitivity of 80% for detecting advanced fibrosis and a specificity of 90% in ruling out advanced fibrosis. ELF was also evaluated in 112 children with NAFLD [69]; the AUROC curve to distinguish among the stages of fibrosis varied from 0.90 to 0.99. In that study, values of ELF from 9.28 to 10.51 had a sensitivity of 88–100% and a specificity of 76–98% to distinguish among the stages.

Imaging assessment of fibrosis in NASH

Conventional ultrasound, CT, and MRI can noninvasively detect hepatic steatosis, and have a good level of accuracy in detecting cirrhosis with portal hypertension. However, they are far less reliable at detecting NASH and the associated stages of fibrosis. The radiologic features of splenomegaly, reversal of hepatic blood flow, change in caudate to right lobe ratio, and hepatic vein narrowing aid the sensitivity of detecting cirrhosis with portal hypertension, but they are less useful in earlier stages of disease. However, new imaging technologies, such as the ultrasonography-based transient elastography (FibroScan), and MRI-based elastography offer promise in determining severity of liver fibrosis associated with NASH.

Ultrasound-based elastography (FibroScan™)

Transient elastography (FibroScan) is a technique whereby shear waves, at a low frequency of 50Hz, are created by a vibrating probe and transducer applied to the skin overlying the liver. The velocity of the propagated wave is correlated with the stiffness or elasticity of the underlying liver; simplistically, the propagated wave travels faster with increasing fibrosis. A pulse–echo ultrasound allows measurement of the wave velocity and the results are presented as kilopascals (kPa). At least 10 validated measures must be obtained. The validity of measurement is assessed by the interquartile range and ratio of successful measurements to unsuccessful measurements which should be over 60%.

The transient elastography technique measures the liver stiffness within a cylinder of 1cm in width and 4cm in length, producing an estimated sampling area that is 100 times greater than biopsy, although the portion of liver sampled is still small. The reproducibility of the technique has been evaluated in a large study including 800 examinations in 200 patients who had heterogeneous liver disease; the intraclass correlation coefficient was 0.98 by two operators [74]. The test is inexpensive and the equipment has a capital cost. The threshold for detecting significant fibrosis varied from 4 to 9kPa in four selected studies that have included patients with mixed causes of chronic liver disease including NAFLD [74–77]. Good diagnostic performance occurs above these critical thresholds. The AUROC curve varied from 0.74 to 0.86 with a sensitivity and specificity of the selected kPa threshold from 68% to 94% and 33 to 85%, respectively. Similar to laboratory tests, FibroScan shows better performance in detecting cirrhotic stage disease.

Few studies have evaluated the performance of FibroScan in staging fibrosis in patients with NAFLD. In the largest study on NAFLD reported to date, 274 patients with NAFLD underwent transient elastography to measure liver stiffness with liver biopsy as the “gold standard”; the AUROC curve was 0.84 for significant fibrosis (F ≥ 2), 0.93 for advanced fibrosis (F ≥ 3) and 0.95 for cirrhosis (F= 4) [78]. A cutoff value of 7.9 kPa was accurate in identifying late stage fibrosis with an associated sensitivity, specificity, PPV and NPV of 91%, 75%, 52% and 97% in identifying fibrosis stage F≥3 [78]. However, FibroScan failed to achieve the 10 validated measures required to assess liver stiffness in 28 of the 274 (10.2%) patients. The AUROC curve for FibroScan to distinguish between advanced (stage

3–4) and non-advanced (stage 0–2) fibrosis nevertheless compared favorably to the AUROC curve for AST/ALT ratio, APRI, FIB-4 score, NAFLD fibrosis score and BARD score in the 246 patients with reliable FibroScan examinations. After "intention to treat" (or to diagnose) analysis, however, a cut-off of 8.7 kPa had a NPV of 89% to exclude advanced fibrosis which did not differ from the NPV for the other previously mentioned simple indices. Furthermore, the PPV to predict the presence of advanced fibrosis was only 48.5% with FibroScan, lower than the NAFLD fibrosis score (61.1%) and the FIB-4 score (59.1%).

Castera et al. investigated the frequency and causes of failure and unreliable results in measuring liver stiffness by FibroScan over a 5-year period, based on more than 13,000 examinations [79]. Failure of fibroscan occurred in 3.1% of all examinations and was independently associated at first examination with BMI greater than 30 kg/m^2; operator experience fewer than 500 examinations; age greater than 52 years; and type 2 diabetes. Unreliable results were obtained in an additional 15.8% of cases and were independently associated with BMI greater than 30 kg/m^2; operator experience fewer than 500 examinations; age greater than 52 years; female sex; hypertension; and type 2 diabetes. The authors concluded that liver stiffness measurements were uninterpretable in nearly one in five cases (19%).

Due to the poor accuracy of the standard M probe of FibroScan in detecting liver fibrosis in overweigh/obese patients [79,80], a new XL probe has been developed and recently evaluated in two large studies [81,82]. In a group of 274 patients with chronic liver disease of different etiology who had a BMI of 28 kg/m^2 or higher, the XP probe provided reliable measurements of liver stiffness in 73% of patients as compared to only 50% of patients with the M probe [81]. Despite the better performance of the X probe in overweight/obese patients, the X probe did not provide reliable measurements of liver stiffness in 27% of the patients, and thus, the X probe was unsuccessful in 1 out 4 overweight/obese patients [81]. Further studies are needed to determine the role of the X probe in overweight/obese patients with NAFLD.

Transient elastography has been evaluated in children with NAFLD [83]. Nobili et al. evaluated the accuracy of the FibroScan in 52 consecutive children with biopsy-proven NAFLD and showed that cutoff values between 7 and 9 kPa predict fibrosis stages 1 or 2, and values ≥9 kPa are associated with the presence of advanced fibrosis [83]. These similarities in the cutoff values suggest that the diagnostic accuracy of FibroScan is independent of the patient's age.

Magnetic resonance elastography (MRE)

In contrast to FibroScan which estimates liver stiffness of a small fraction of the liver, magnetic resonance elastography (MRE) estimates the average degree of liver fibrosis throughout most of the liver parenchyma by assessing the propagation of mechanical waves through the tissue. First, shear waves are generated in the liver tissue by a driver (pneumatic or electromechanical) attached to the abdominal wall. Magnetic resonance images are then obtained depicting the propagated shear waves, and finally, images of the shear waves are analysed and used to generate quantitative maps of tissue stiffness, referred to as elastograms [84]. As the entire liver can be sequenced, the area of sampling is greatly increased and the heterogeneous distribution of fibrosis is more commonly appreciated. In a study by Huwart et al., MRE performed better than FibroScan and AST-to-platelet ratio index (APRI) in 141 patients with chronic liver disease of various etiologies [85]. The AUROC curves were significantly greater for distinguishing any stage of fibrosis using MRE. The technical success rate of MRE was significantly higher than that of FibroScan (94% vs 84%). The AUROC curve of MRE (0.994 for F≥2; 0.985 for F≥3; 0.998 for F=4) were significantly higher than those of FibroScan, APRI, and the combination of FibroScan

and APRI. The study demonstrated that MRE has a higher technical success rate than FibroScan and a better diagnostic accuracy than FibroScan and APRI for staging liver fibrosis. In a tertiary center in Asia, MRE was shown to increase systematically along with fibrosis stage in a cohort of 60 patients – 55 with chronic hepatobiliary diseases and 5 living related liver donors [86]. With a shear stiffness cut-off of 3.05 kPa, the predicted sensitivity and specificity for differentiating liver fibrosis (F ≥2) from mild fibrosis (F1) were 89.7% and 87.1%, respectively. Impressively, MRE was able to discriminate between patients with with severe fibrosis (F3) and those with liver cirrhosis (sensitivity 100%, specificity 92.2%), with a shear stiffness cut-off value of 5.32 kPa.

Rather intriguing are the recent findings showing that MRE is useful in helping to identify patients with steatohepatitis, even prior to the onset of fibrosis [87]. In this retrospective study of 58 NAFLD patients, hepatic stiffness had high accuracy in discriminating patients with NASH from those with simple steatosis (AUROC = 0.93, sensitivity 94%, specificity 73% by using a threshold of 2.74 kPa). NAFLD patients with inflammation (NASH) but no fibrosis have greater liver stiffness than those with simple steatosis and lower mean stiffness than those with fibrosis [87].

Despite these encouraging results, there are still issues of concern with MRE techniques. These include the increased acquisition time of scanning, the costs of the equipment, the expertise in analysis, and standardized thresholds of measurement. Nevertheless, it hold great promise as a noninvasive alternative to liver biopsy in staging fibrosis in NASH, but more studies are needed to determine the role of MRE to distinguish between simple steatosis and NASH.

Other imaging modalities

Other modalities that are still being evaluated and may be more widely utilized in the future include acoustic radiation force impulse imaging (ARFI) to assess liver fibrosis [88,89], optical analysis of CT-generated images to predict fibrosis stage and distribution [90], and diffusion-weighted MRI for the diagnosis of liver fibrosis and cirrhosis [91]. These imaging modalities need to be evaluated in patients with NAFLD to determine their accuracy in diagnosing and staging this disease.

Summary

NAFLD affects a substantial proportion of the population worldwide. Only a minority of subjects who have the condition develop liver-related complications. Predicting which patients will develop progressive disease is problematic. Currently, there is no available noninvasive test demonstrated to be simple, reproducible, and valid for disease staging in patients with NAFLD. Liver biopsy remains the gold standard investigation to distinguish between patients with NASH and those without NASH or bland steatosis, and to determine disease prognosis based on fibrosis staging. Plasma levels of cytokeratin-18 is the most promising but imperfect non-invasive test to distinguish NASH from bland steatosis. Several simple scoring formulas that combine routinely measured clinical and laboratory variables as well as some proprietary serum panels are currently available to predict severity of liver fibrosis. Both the proprietary panels and simple indices seem equally accurate. Liver stiffness measured by ultrasound-based elastography or FibroScan may detect occult cirrhosis but the technique is often unreliable in patients with NAFLD and obesity. In addition, FibroScan is not superior to simple indices in predicting severity of liver fibrosis in NAFLD. Magnetic resonance-based elastrography or MRE has a high sensitivity and specificity for diagnosing and staging liver fibrosis in patients with chronic liver disease of various etiologies. However, further work is needed to determine the accuracy of MRE in diagnosing and staging liver fibrosis in patients with NAFLD and in distinguishing between

patients with bland steatosis and those with NASH. MRE is not yet widely available and cost and expertise in interpretation is likely to be a limiting factor to its use. In the future, indirect and direct serum markers of NASH and liver fibrosis alone or in combination with imaging modalities will likely be increasingly translated into simple diagnostic kits and algorithms for viable, noninvasive alternatives to liver biopsy.

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1. NAFLD affects a substantial proportion of the population worldwide.
2. Only a minority of subjects who have the condition develop liver-related complications.
3. Predicting which patients will develop progressive disease is problematic.
4. Currently, there is no available noninvasive test demonstrated to be simple, reproducible, and valid for disease staging in patients with NAFLD.
5. Liver biopsy remains the gold standard investigation to distinguish between patients with NASH and those without NASH or bland steatosis, and to determine disease prognosis based on fibrosis staging.

Table 1

Noninvasive diagnosis of NASH

Author (ref)	n	Variables	Cutoff	AUROC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Poynard et al. (29)	257, 97, 383	Age, gender, BMI, triglycerides, cholesterol, α-2 macroglobulin, gamma-GT, AST, ALT, haptoglobin, apolipoprotein A1, total bilirubin	ND	0.79	29	98	91	71
Palekar et al. (30)	80	Age ≥50, female, AST≥45, AST/ALT ratio ≥0.8, BMI ≥30, hyaluronate ≥55 mcg/L	≥ 3	0.76	74	66	68	71
Shimada et al. (31)	85	Serum adiponectin, type IV collagen 7s level, HOMA-IR	ND	ND	94	74	94	74
Feldstein et al. (32,33)	39 139 (validation)	CK-18 levels	395 U/L 250 U/L	0.93 0.83 (95% CI 0.75, 0.91)	87 0.75 (95% CI 0.64, 0.83)	100 0.81 (95% CI 0.61, 0.93)	100	86

FOOTNOTE: NASH, nonalcoholic steatohepatitis; AUROC, area under the receiver operating characteristics curve. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase

Table 2

Routine laboratory and clinical predictors of advanced fibrosis in patients with NAFLD

Author (ref)	n	Patient population	Risk factors	Odds ratio (95% CI)
Angulo et al. (38)	144	NASH	Age ≥ 45 years	5.6 (1.5, 21.7)
			Obesity (BMI > 30 kg/m^2)	4.3 (1.4, 13.8)
			Diabetes	3.5 (1.2, 9.8)
			AST/ALT ratio > 1	4.3 (1.5, 12)
Marceau et al. (50)	551	Bariatric surgery patients	Age	NA
			Diabetes	NA
			Waist-hip ratio	NA
			BMI	NA
Ratziu et al. (48)	93	Overweight, raised liver tests	Age ≥ 50 years	14.1 (3.7, 54.0)
			BMI ≥ 28 kg/m^2	5.7 (1.6, 20.0)
			Triglyceride ≥ 1.7 mmol/L	5.0 (1.4, 17.0)
			ALT ≥ 2 × ULN	4.6 (1.3, 16.0)
Dixon et al. (49)	105	Bariatric surgery patients	Hypertension	NA
			ALT > 40 IU/L	NA
			Insulin resistance > 5.0	NA
Angulo et al. (51)	733	NAFLD	Age(years)	1.04 (1.01, 1.07)
			BMI (kg/m^2)	1.10 (1.04, 1.16)
			IFG/Diabetes	3.12 (1.77, 5.51)
			AST/ALT ratio	2.70 (1.33, 5.62)
			Platelet count (×10^9/L	0.987 (0.98, 0.99)
			Albumin (g/dl)	0.51 (0.25, 1.05)
Harrison et al. (52)	827	NAFLD	BMI ≥ 28 kg/m^2	2.4 (1.2, 4.8)
			AST/ALT ratio ≥ 0.8	9.3 (6.3, 13.6)
			Diabetes	4.0 (2.8, 5.7)
Miyaaaki et al. (53)	182	NAFLD	Female gender	4.60 (1.68, 12.58)
			Age ≥ 60	2.73 (1.23, 5.94)
			Type 2 diabetes	3.43 (1.48, 7.92)
			Hypertension	3.58 (1.63, 7.90)

BMI: body mass index; NA: not available; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ULN: upper limit of normal

Table 3

Predicting advanced fibrosis (F3-4) using routine clinical and laboratory variables in NAFLD

Predictive score (ref)	n	Variables/Formula	AUROC (95% CI)	Cut-off points	PPV (%)	NPV (%)
NAFLD fibrosis score (51)	733	−1.675 + 0.037 × age [yrs] + 0.094 × BMI [kg/m^2] + 1.13 × IFG/diabetes [yes=1, no=0] + 0.99 × AST/ALT ratio - 0.013 × platelet count [×10^9/l] - 0.66 × albumin [g/dl]	0.88 (0.85, 0.92)	≤ −1.455 ≥ 0.676	56 90	93 85
BARD score (52)	827	BMI ≥28 = 1 AST/ALT ratio ≥0.8 = 2 Diabetes = 1 Score ≥ 2, odds ratio for adv. fibrosis = 17	ND	<2 points		96
FIB-4 score (56)	541	Age [yrs] × AST [U/L]/platelet [×10^9/l] × ALT[U/L]	0.80 (0.76, 0.85)	≤ 1.30 ≥ 2.67	43 80	90 83

BMI: body mass index; IFG: impaired fasting glucose; AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 4

Serum markers of fibrogenesis and clinical predictors of advanced fibrosis (F3-4) in patients with NAFLD

Author [re]	n	Serum marker	AUROC	Sensitivity (%)	Specificity (%)
Suzuki et al. [63]	79	Hyaluronic acid > 46.1 ng/ml	0.89	85.0	79.7
Sukugaya et al. [64]	112	Hyaluronic acid ≥ 50 ng/ml	0.80	68.8	82.8
		Type IV collagen 7S ≥ 5 ng/ml	0.82	81.3	71.4
Palekar et al. [65]	80	Hyaluronic acid > 45.3 ng/ml	0.88	85.7	80.3
Kaneda et al. [70]	148	Hyaluronic acid > 42 ng/ml		100.0	89.0
Santos et al. [66][a]	30	Hyaluronic acid > 24.6 ng/ml	0.73	82.0	68.0
		Type IV collagen > 145 ng/ml	0.80	64.0	89.0
		Laminin > 282 ng/ml	0.87	82.0	89.0
Ratziu et al. [67]	267	Fibrotest 0.30	0.88	92.0	71.0
		Fibrotest 0.70	0.88	25.0	97.0
Guha et al. [68]	192	ELF score = −7.412 + (ln(HA)*0.681) + (ln(P3NP)*0.775) + (ln(TIMP1)*0.494) [b] ELF = 0.3576	0.93	80	90
Nobili et al. [69]	112	ELF (different cut-of values)	0.90–0.99	88–100	76–98

AUROC: area under the receiver operating characteristics curve. The AUROC is used to distinguish between patients with and without advanced fibrosis.

ELF: enhanced liver fibrosis panel; TIMP1: tissue inhibitor of metalloproteinase 1.

[a] Predicting the presence of fibrosis versus the absence of fibrosis

[b] An ELF score of 0.3576 has a sensitivity of 80% in detecting advanced fibrosis and a specificity of 90% in ruling out advanced fibrosis.