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Non-invasive Testing for NASH and NASH with Advanced Fibrosis: Are We There Yet?

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent etiology of chronic liver disease in America. NAFLD can be broadly classified in two subtypes: nonalcoholic fatty liver (NAFL), which is generally considered a benign condition with negligible risk of progression to cirrhosis, and nonalcoholic steatohepatitis (NASH), which is generally considered to be progressive with substantial risk of progression to cirrhosis. Additionally, recent studies suggest the odds of liver mortality increases amongst NASH patients with advanced fibrosis (bridging fibrosis $\pm$ cirrhosis). Liver biopsy examination is the current gold standard to accurately discriminate between NAFL vs. NASH as well as diagnose advanced fibrosis. However, due to its invasive nature, risk of bleeding (and even rarely death), prohibitive cost, and sampling error, liver biopsies are imperfect for diagnosis and monitoring of NAFLD. As a result, noninvasive biomarkers that can accurately detect NASH and advanced fibrosis without biopsy are needed. This article will discuss the most novel noninvasive biomarkers in diagnosing NASH and advanced fibrosis.

Keywords

advanced fibrosis; nonalcoholic fatty liver disease; noninvasive biomarker; nonalcoholic steatohepatitis

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Compliance with Ethics Guidelines

Conflict of Interest

Ahilan Arulanandan declares no conflict of interest.

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.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent etiology of chronic liver disease in America and it is being increasingly recognized worldwide.^{1, 2} NAFLD is defined as hepatic steatosis in the setting of minimal alcohol use plus no other identifiable secondary cause of hepatic steatosis.^{3, 4} NAFLD can be broadly classified into two subtypes: nonalcoholic fatty liver (NAFL), which is generally considered a benign condition with negligible risk of progression to cirrhosis, and nonalcoholic steatohepatitis (NASH), which is generally considered to be harmful with substantial risk of progression to cirrhosis. NASH characteristics include: lobular inflammation, hepatic steatosis typically near the central veins, and classic ballooning $\pm$ peri-sinusoidal fibrosis. Non-fibrotic NASH can further progress to advanced fibrosis, cirrhosis, and hepatocellular carcinoma.⁵⁻⁷ Among NAFLD patients, those with NASH have greater rates of metabolic syndrome, cardiac disease, and increased mortality than those with NAFL.⁸⁻¹⁰ Additionally, a diagnosis of NASH with advanced fibrosis portends an even poorer prognosis.^{1, 10, 11} Due to these prognostic implications, discriminating between NAFL vs. NASH and advanced fibrosis is a critically important clinical question.

The current gold standard to differentiate between NAFL vs. NASH and advanced fibrosis is via liver biopsy. However, due to its invasive nature, risk of bleeding (and even rarely death), prohibitive cost, and sampling error, liver biopsies are imperfect for diagnosis and monitoring of NAFLD.¹² Therefore, there is a pressing need for noninvasive diagnostic biomarkers to accurately identify patients with progressive etiologies of NAFLD, such as NASH and advanced fibrosis.¹³⁻¹⁵ Multiple reviews in the past decade have discussed noninvasive biomarkers for NAFLD so this article will concentrate on the most novel noninvasive biomarkers for NASH and advanced fibrosis in the past few years of literature.

Serum Biomarkers

Markers of cellular injury: CK-18, Fas ligand, and Procollagen III

Hepatocyte death is an indicator of NAFLD progression into more advanced stages of fatty liver disease. Utilizing the aforementioned pathology, serum biomarkers have demonstrated potential in predicting NASH and advanced fibrosis. Fragments of cytokeratin 18 (CK-18) have been shown to be increased in NASH and advanced fibrosis versus NAFL patients.¹⁶ CK-18, a protein found in the liver, can be quantified with a monoclonal antibody M30 via an ELISA based assay. A newer ELISA based assay, M65, can detect both CK-18 fragments as well as uncleaved CK-18. Measuring the total amount of CK-18 via the M65 assay has also demonstrated potential in liver disease patients by predicting levels of steatosis and fibrosis.¹⁷ A recent 2014 report of 424 subjects by Cusi *et al.*¹⁸ demonstrated that CK-18 has strong accuracy in identifying both fatty liver disease and fibrosis. However, the study also found that CK-18 is of limited value in identifying NASH. Furthermore, Vuppalanchi and colleagues examined the clinical utility of change in CK-18 levels as a predictor or correlate of treatment response in NASH in the PIVENs trial.¹⁹ CK-18 performed poorly as predictor of treatment response and was not better than serum alanine aminotransferase (ALT) in predicting response to treatment in NASH. Therefore, CK-18 has potential to be included into a panel of liver injury biomarkers in NAFLD, but would not by itself be

helpful in non-invasively diagnosing NASH or advanced fibrosis, and is not accurate for predicting treatment response in NASH trials.

Another serum biomarker, the Fas ligand, is a transmembrane protein in the liver that induces apoptosis and has demonstrated increased levels in NASH vs. NAFL.²⁰ A 2011 paper by Tamimi *et al.*²¹ used both CK-18 and Fas levels to create an algorithm that accurately predicted NASH vs. NAFL with a high AUROC in the training (0.93) and validation (0.79) sets. The Fas ligand has also been demonstrated to be associated with hepatic fibrosis.²² However, its prognostic use in identifying advanced fibrosis or identifying NASH patients without fibrosis is less known. Further research is needed to validate the Fas ligand's role as a biomarker in NASH and advanced fibrosis. Lastly, procollagen III (PIIINP) is a peptide that is cleaved during collagen turnover and is a known marker of tissue repair and fibrosis.²³ A 2013 study by Tanwar *et al.*²⁴ identified PIIINP as an accurate biomarker in discriminating between NAFL, NASH, and advanced fibrosis. They also demonstrated that, in non-fibrotic NAFLD, PIIINP could differentiate between NASH and NAFL. However, PIIINP's ability to discriminate between the differing degrees of fibrosis was not significant. Therefore, further trials are required prior to this marker's clinical application.

Inflammatory markers: Adipokines and Ferritin

The chronic inflammatory state in obese NAFLD patients has long been thought to advance NAFL to NASH and advanced fibrosis.^{25, 26} Cytokines such as tumor necrosis factors, interleukins, and transforming growth factors have demonstrated associations with progression of NAFLD.²⁷ Adipose tissue contains adipokines, essentially cytokines of adipose tissue, which help coordinate the hyperinflammatory state that may drive fatty liver disease progression. A 2012 study by Machado and colleagues used this pathology to identify significant adipokines in a trial of NAFLD patients with morbid obesity.²⁸ These patients had elevated levels of leptin, adiponectin, and ghrelin. These three factors were measured and incorporated into an algorithm that demonstrated AUROC of 0.79 for identifying NASH vs. NAFL in NAFLD patients. However, the role of adipokines in discriminating between stages of fibrosis was less clear.

Another well-known inflammatory marker, serum ferritin, was previously demonstrated to be increased in advanced fibrosis and NASH.²⁹ A 2011 study of NAFLD patients by Manousou *et al.*³⁰ found ferritin to be an independent predictor of NASH. Additionally, a 2012 study by Kowdley *et al.*²⁹ reported that ferritin was an independent risk factor of advanced fibrosis in NAFLD. However, a more recent study of 1,201 Japanese patients by Yoneda and colleagues found ferritin to be of low diagnostic use in the detection and differentiation of stages of fibrosis due to multiple confounders.³¹ As ferritin is elevated in a variety of chronic disease and inflammatory states, it is likely too nonspecific to have significant clinical utility in diagnosing NASH or advanced fibrosis.

Oxidative stress

Oxidative stress is another mechanism that accelerates NAFL progression to NASH and fibrosis.^{32, 33} Markers of oxidative stress such as glutathione peroxide dismutase, superoxide

dismutase, and oxidized low-density lipoprotein have demonstrated utility in predicting NASH and advanced fibrosis.^{34, 35} Elevation of the oxidation products of linoleic and arachidonic acid have also been demonstrated to accurately discriminate between NAFL and NASH in NAFLD patients.^{36, 37} In addition, recent research on vitamin E's ameliorative effects on NASH progression provides further evidence of the role of oxidation in disease progression.³⁸ Though this research has been promising, further elucidation and trials of the ideal markers of oxidative stress are needed before clinical applications can occur.

Lipotoxicity

Lipotoxicity is also thought to be one of the mechanisms underlying progression of NAFL into NASH and advanced fibrosis. It was recently demonstrated that plasma eicosanoids as well as metabolites of polyunsaturated fatty acids (PUFA) could help identify NAFL vs. NASH.³⁹ In this pilot study, 11, 12-dihydroxy-eicosatrienoic acid (11,12-diHETrE) was identified as the most specific PUFA to differentiate between NAFL and NASH (AUROC of 1). The authors also identified a comprehensive panel, which included 20-carboxy arachidonic acid (20-COOH AA) and 13, 14-dihydro-15-keto prostaglandin D₂ (dkPGD2), which also had an AUROC of 1. These findings, although extremely encouraging, require extensive validation in larger future studies.

In summary, multiple trials of serum biomarkers have demonstrated utility in discriminating NASH and advanced fibrosis from NAFL. Although many studies have been promising, their lack of commercial availability and limited number of validated trials demonstrate that further analyses are needed prior to widespread clinical use.

Clinical predictor models

In order to better identify the risk of NASH and advanced fibrosis, various clinical predictor models have been proposed over the past several decades. Well-validated predictors include obesity,^{40, 41} age,^{40–43} hypertension,^{44, 45} and hypertriglyceridemia,⁴⁰ but their accuracy in diagnosing NASH or staging fibrosis is poor. As clinical predictor models are a fast, easy method for general practitioners to identify at-risk NAFLD patients, their development is crucial to management of this disease.

A common method frequently used in practice is the NAFLD fibrosis score. Developed in 2007 by Angulo *et al.*,⁴⁶ the authors determined that hyperglycemia, albumin, age, body mass index (BMI), platelet count, and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio were solitary predictors of advanced fibrosis. They identified that, amongst their cohort of over 700 individuals, 75% would not have been biopsied with correct prediction in 90% of them. They summarized that the NAFLD fibrosis score can accurately identify whether advanced fibrosis is present in NAFLD. A meta-analysis in 2011 that included 13 studies and over 3,000 patients found that the NAFLD fibrosis score had a high AUROC (0.85) in prediction of advanced fibrosis.⁴⁷ The NAFLD fibrosis score is widely used due to its ease of use and the accessibility of clinical tests.

Another popular model, the BARD score, is composed of BMI, diabetes, and AST/ALT ratio, and is an even simpler method to determine fibrosis risk.⁴⁸ The BARD score was

created by a retrospective analysis of 827 patients in 2008 that demonstrated a high negative predictive value (NPV) of 96%. With easily identifiable risk factors (BMI, diabetes, and AST/ALT ratio) and a simple method of ruling out patients, the BARD score is popular amongst general practitioners. However, its low positive predictive value (42%) caused many patients without NASH or fibrosis to be included as high-risk patients. This led to a retrospective 2013 study of 107 patients by Lee *et al.*⁴⁹ that added International Normalized Ratio (INR) to the BARD score to create a BARDI score. When compared to BARD, BARDI demonstrated an improved PPV (51.0%) and kept a similar NPV (100%).

More recent clinical predictor models have been created in the last few years with promising findings. The FIB-4 index (AST, ALT, platelets, age) has demonstrated superiority in a validation study to BAAT, ELF, AAR, AP index, APRI, BARD, and NAFLD fibrosis score.⁵⁰ The OxNASH score, which uses BMI, age, AST, and 13-(S)-hydroxyoctadecadienoic acid (13-HODE), has demonstrated accuracy as a prognostic indicator of NASH and staging of fibrosis.³⁷ Finally, a 2014 trial by Cao *et al.*⁵¹ combined CK-18, ALT, platelets, and triglycerides to create a scoring system that had significant positive correlation with staging fibrosis. Predicting NASH in their cohort had an AUROC of 0.920, although this has not yet been externally validated.

In summary, clinical predictor tools have demonstrated strong associations with predicting NASH and staging fibrosis. As their components are generally inexpensive and readily available, they are useful tools for practitioners in initiating management of fatty liver disease. Table 1a summarizes clinical predictors and biomarkers for identifying NASH, while Table 1b summarizes clinical predictors and biomarkers for identifying advanced fibrosis.

Imaging tools

Recent innovations in imaging are demonstrating potential to help diagnose NASH and advanced fibrosis. Although there are no current imaging techniques that directly measure hepatic fibrosis, there are tests that can indirectly detect fibrosis via tissue stiffness, perfusion, and texture, amongst others. The leading biomarker among these indirect tests is liver stiffness, also called elasticity. Tissue elasticity is simply the relative softness of a tissue. As liver fibrosis increases due to collagen deposition and parenchymal rigidity, its elasticity decreases. The imaging techniques that display elastic properties of tissue are collectively called “elastography” and will be discussed below.

Transient elastography (TE) measures liver elasticity via a specialized bedside ultrasound. A TE probe vibrates the skin to generate a “shear wave” that moves through the skin into hepatic tissue. A specialized transducer identifies the speed of the shear wave to determine elasticity. It is a quick, painless, and easy to perform test that has demonstrated accuracy in both identifying and staging fibrosis.^{52–54} However, TE has its limitations. It is an operator dependent ultrasound, needs different probes for obese or pediatric patients, and is not reliable in patients with ascites because the shear wave is distorted by fluid.

Due to the limitations of TE, specifically in obese or ascites patients, another ultrasound method, acoustic radiation force impulse elastography (ARFI), has gained popularity. ARFI

has demonstrated ability to diagnose fibrosis and separate more advanced fibrosis (F3-F4) from less advanced fibrosis (F0-F2) with similar accuracy to TE.⁵⁵⁻⁵⁸ ARFI uses a conventional US probe that produces an acoustic pulse to mechanically excite tissue. The tissue creates a propagating shear wave opposite to the area of excitation and measurement of resulting displacement is taken. Its mechanism of action is an improvement on TE because the acoustic energy pulse doesn't change with obesity or ascitic fluid. A recent meta-analysis of 1163 patients with biopsy-proven fibrosis demonstrated that inability to record consistent readings occurred three times more often with TE compared to ARFI.⁵⁶ Additionally, ARFI can be focused on specific regions of interest to measure varying depths in distinct areas of the liver. Lastly, a full sonographic evaluation of the liver can be done at the same time as ARFI, which is not possible with TE.

Another US technique, shear wave elastography (SWE), uses acoustic pulses like ARFI to transmit energy into the tissue of interest. The tissue's elasticity is measured by how fast the resulting shear wave travels through the tissue and a quantitative estimate of tissue stiffness is calculated. Like ARFI, SWE has real-time imaging capabilities and visualizes images in 2D, which cannot be done with the 1D model of TE. Additionally, SWE uses a higher frame-rate B-mode image which theoretically may be more accurate in staging fibrosis.⁵⁹ Multiple trials have demonstrated SWE to have at least similar accuracy to TE and ARFI in staging fibrosis.⁶⁰⁻⁶² Other studies, however, have found that SWE couldn't accurately discriminate between stages of fibrosis.⁶³

Each of the US techniques mentioned (TE, ARFI, and SWE) have demonstrated potential accuracy in identifying and discriminating between stages of fibrosis. However, there are no studies that unequivocally demonstrate the superiority of one method and they each have various pros and cons (see table 2). Additionally, current US techniques have not demonstrated a consistent ability to accurately diagnose NASH in patients without fibrosis. As up to 40% of NASH patients do not have any fibrosis, these imaging modalities are limited in separating NAFL from NASH without fibrosis.^{64, 65}

The final imaging modality we will discuss, magnetic resonance elastography (MRE), resolves some of the problems faced by US but has deficiencies of its own. MRE records shear waves to generate a stiffness map of the liver via placing regions of interest.⁶⁶ MRE can measure in two or three planes, analyze a larger surface area of the liver (or the entire liver) than US-based modalities, and is not limited by ascites and/or obesity. Studies have suggested MRE has higher accuracy compared to TE and APRI for discriminating fibrosis.⁶⁷⁻⁷⁰ Additionally, a 2011 study by Chen *et al.*⁷¹ demonstrated that MRE can accurately identify individuals with NASH prior to fibrosis and had an AUROC of 0.93 for differentiating between NAFL and NASH. However, no further publications have reached this conclusion, so validation is still needed.

A 2014 prospective trial of 117 individuals with NAFLD found that 2D-MRE successfully discriminated between less (stage 0-2) and more advanced fibrosis (stage 3-4) with an AUROC of 0.924.⁷⁰ This single center study showed that MRE has potential as a non-invasive tool in predicting and diagnosing advanced fibrosis in NAFLD patients. However, multicenter studies are imperative in further validating these findings. Additionally, current

studies such as the MOZART trials are demonstrating how combining imaging modalities (in this case a co-localization of MRI-PDFF (proton density fat fraction) derived fat-maps and MRE-derived stiffness-maps of the liver) before and after treatment can be used to non-invasively assess treatment response in NASH.⁷²

In summary, innovations in imaging have demonstrated accuracy in diagnosing and discriminating between stages of fibrosis but are currently suboptimal in differentiating between NAFL and NASH without fibrosis. New imaging tools, such as quantitative US (QUS) and MRI-PDFF, are continually being developed and are exhibiting value in NAFLD research.^{73, 74} The utility of US, 2D and 3D MRE, or a combination of multiple imaging modalities to assess treatment response in NASH and anti-fibrotic trials remains to be assessed.

Breath tests

As metabolites of oxidative products are often elevated in chronic inflammatory disease, studying these metabolites may have utility in NAFLD. Using this theory, a 2013 study by Verdam *et al.*⁷⁵ analyzed respired breath of 65 subjects. They found that NASH could be accurately predicted by 3 volatile organic compounds (VOC): n-tridecane, 3-methyl-butanonitrile, and 1-propanol with an AUROC of 0.77 in differentiating NASH from non-NASH in NAFLD patients.

Another breath test trial, by Banasch and colleagues,⁷⁶ utilized mitochondrial dysfunction in the pathology of fatty liver disease. They measured levels of (13)C-methionine, which is an indicator of mitochondrial function in the liver. They found that (13)C-methionine accurately predicted levels of fatty liver disease activity as decreased exhalation of the compound correlated with increased rates of fibrosis and NASH. Amongst the total cohort, AUROC for NASH was 0.87 compared to 0.83 for patients with mild or no fibrosis.

Although relatively new, breath tests are demonstrating potential to be useful in predicting NASH and fibrosis. Further trials and validation trials are required.

Genetic factors

The study of genetic factors in NAFLD is a rapidly growing field. Multiple Genome Wide Association Studies (GWAS) are demonstrating the roles that various genes play in increasing risk of fatty liver disease. For example, patatin-like phospholipase 3 (PNPLA3), has been demonstrated to be a genetic risk factor for presence of hepatic steatosis and presence of NASH.⁷⁷⁻⁷⁹ The PNPLA3 gene has also recently shown associations with increased risk of fibrosis and hepatocellular carcinoma.^{80, 81} Another gene, apolipoprotein C3 (APOC3), was demonstrated in a 2010 study by Peterson and colleagues to be associated with NAFLD.⁸² However, subsequent trials of 470 patients by Verrijken *et al.*⁸³ and Sentinelli *et al.*⁸⁴ found that APOC3 had no association with NAFLD.

More recently, variants of the TM6SF2 genotype have been correlated with higher rates of NASH and advanced fibrosis.^{85, 86} Interestingly, Dongiovanni and colleagues demonstrated that patients with the E167K variant of TM6SF2 had a decreased incidence of

cardiovascular events despite increased likelihood of NASH and advanced fibrosis. Variants of TM6SF2 are thought to inhibit hepatic excretion of very low-density lipoprotein (VLDL), thereby increasing fat in the liver but decreasing circulating VLDL. There are several other SNPs that have been associated with NAFLD and due to space limitations we were not able to include all genetic association studies in this section, and would refer the readers to previous reviews on the topic for further details.^{87, 88}

Conclusions

Advancements in noninvasive testing for NASH and advanced fibrosis continue to demonstrate promise in helping diagnose and manage fatty liver disease. Clinical predictor models are already in widespread use and often direct practitioners away or towards a liver biopsy. Serum biomarkers, genetic tests, and breath tests have shown strong potential to change how we screen and diagnose patients in the near future. Finally, the emergence of novel imaging tools will conceivably change clinical practice in the coming years. Although pathological exam of liver tissue is still the gold standard in diagnosis of NASH and advanced fibrosis, the emergence and continued improvement of various noninvasive biomarkers are altering how we predict and manage fatty liver disease.

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References

** denotes reference of major importance

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1. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology*. 2005; 129:113–21. [PubMed: 16012941]
2. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, et al. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int*. 2006; 26:856–63. [PubMed: 16911469]
3. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005; 41:1313–21. [PubMed: 15915461]
- **4. Singh S, Allen AM, Wang Z, et al. Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A Systematic Review and Meta-analysis of Paired-Biopsy Studies. *Clin Gastroenterol Hepatol*. 2014 This was the first study to document fibrosis progress rate in NAFLD and NASH.
5. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology*. 2012; 55:2005–23. [PubMed: 22488764]

6. Stepanova M, Rafiq N, Makhlof H, et al. Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD). *Dig Dis Sci*. 2013; 58:3017–23. [PubMed: 23775317]
7. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology*. 2011; 140:124–31. [PubMed: 20858492]
8. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol*. 2008; 49:608–12. [PubMed: 18682312]
9. Söderberg C, Stål P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology*. 2010; 51:595–602. [PubMed: 20014114]
10. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology*. 2006; 44:865–73. [PubMed: 17006923]
11. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology*. 1994; 19:1513–20. [PubMed: 8188183]
12. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med*. 2001; 344:495–500. [PubMed: 11172192]
13. Mato JM, Lu SC. Where are we in the search for noninvasive nonalcoholic steatohepatitis biomarkers? *Hepatology*. 2011; 54:1115–7. [PubMed: 21898494]
14. Ratziu V, Cadranet JF, Serfaty L, et al. A survey of patterns of practice and perception of NAFLD in a large sample of practicing gastroenterologists in France. *J Hepatol*. 2012; 57:376–83. [PubMed: 22521354]
15. Corey KE, Lai M, Gelrud LG, et al. Non-high-density lipoprotein cholesterol as a biomarker for nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2012; 10:651–6. [PubMed: 22330232]
16. Alkhouri N, Carter-Kent C, Feldstein AE. Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications. *Expert Rev Gastroenterol Hepatol*. 2011; 5:201–12. [PubMed: 21476915]
17. Joka D, Wahl K, Moeller S, et al. Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis. *Hepatology*. 2012; 55:455–64. [PubMed: 21993925]
18. Cusi K, Chang Z, Harrison S, et al. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol*. 2014; 60:167–74. [PubMed: 23973932]
- *19. Vuppalanchi R, Jain AK, Deppe R, et al. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2014; 12:2121–30. e1–2. This study demonstrated keratin 18 was not a good predictor of treatment response in NASH patients and did not perform any better than ALT. [PubMed: 24846279]
20. Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology*. 2003; 125:437–43. [PubMed: 12891546]
21. Tamimi TI, Elgouhari HM, Alkhouri N, et al. An apoptosis panel for nonalcoholic steatohepatitis diagnosis. *J Hepatol*. 2011; 54:1224–9. [PubMed: 21145805]
22. Wang K. Molecular mechanisms of hepatic apoptosis. *Cell Death Dis*. 2014; 5:e996. [PubMed: 24434519]
23. Khan S, Subedi D, Chowdhury MM. Use of amino terminal type III procollagen peptide (P3NP) assay in methotrexate therapy for psoriasis. *Postgrad Med J*. 2006; 82:353–4. [PubMed: 16679477]
24. Tanwar S, Trembling PM, Guha IN, et al. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. *Hepatology*. 2013; 57:103–11. [PubMed: 22930399]
25. Wieckowska A, Papouchado BG, Li Z, et al. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2008; 103:1372–9. [PubMed: 18510618]

26. Wieckowska A, Papouchado BG, Li Z, et al. Role of interleukin-6 in human nonalcoholic steatohepatitis. Response to Dogru et al. *Am J Gastroenterol*. 2009; 104 author reply 788.
27. Mirza MS. Obesity, Visceral Fat, and NAFLD: Querying the Role of Adipokines in the Progression of Nonalcoholic Fatty Liver Disease. *ISRN Gastroenterol*. 2011; 2011:592404. [PubMed: 21991518]
28. Machado MV, Coutinho J, Carepa F, et al. How adiponectin, leptin, and ghrelin orchestrate together and correlate with the severity of nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2012; 24:1166–72. [PubMed: 22735605]
29. Kowdley KV, Belt P, Wilson LA, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology*. 2012; 55:77–85. [PubMed: 21953442]
30. Manousou P, Kalambokis G, Grillo F, et al. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. *Liver Int*. 2011; 31:730–9. [PubMed: 21457446]
31. Yoneda M, Thomas E, Sumida Y, et al. Clinical Usage of Serum Ferritin to Assess Liver Fibrosis in Patients with Nonalcoholic Fatty Liver Disease: Proceed with Caution. *Hepatol Res*. 2014
32. Oliveira CP, da Costa Gayotto LC, Tatai C, et al. Oxidative stress in the pathogenesis of nonalcoholic fatty liver disease, in rats fed with a choline-deficient diet. *J Cell Mol Med*. 2002; 6:399–406. [PubMed: 12417056]
33. Videla LA, Rodrigo R, Araya J, et al. Oxidative stress and depletion of hepatic long-chain polyunsaturated fatty acids may contribute to nonalcoholic fatty liver disease. *Free Radic Biol Med*. 2004; 37:1499–507. [PubMed: 15454290]
34. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2011; 33:525–40. [PubMed: 21198708]
35. Adams LA, Feldstein AE. Non-invasive diagnosis of nonalcoholic fatty liver and nonalcoholic steatohepatitis. *J Dig Dis*. 2011; 12:10–6. [PubMed: 21091933]
36. Feldstein AE, Lopez R, Tamimi TA, et al. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *J Lipid Res*. 2010; 51:3046–54. [PubMed: 20631297]
37. Alkhoury N, Berk M, Yerian L, et al. OxNASH score correlates with histologic features and severity of nonalcoholic fatty liver disease. *Dig Dis Sci*. 2014; 59:1617–24. [PubMed: 24464211]
38. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. 2010; 362:1675–85. [PubMed: 20427778]
- **39. Loomba R, Quehenberger O, Armando A, et al. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res*. 2015; 56:185–92. This study identified polyunsaturated fatty acid metabolites that could differentiate NAFL from NASH. [PubMed: 25404585]
40. Ratzliff V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. *Gastroenterology*. 2000; 118:1117–23. [PubMed: 10833486]
41. Angulo P, Keach JC, Batts KP, et al. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology*. 1999; 30:1356–62. [PubMed: 10573511]
42. Marceau P, Biron S, Hould FS, et al. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab*. 1999; 84:1513–7. [PubMed: 10323371]
43. Noureddin M, Yates KP, Vaughn IA, et al. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. *Hepatology*. 2013; 58:1644–54. [PubMed: 23686698]
44. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology*. 2001; 121:91–100. [PubMed: 11438497]
45. Papadia FS, Marinari GM, Camerini G, et al. Liver damage in severely obese patients: a clinical-biochemical-morphologic study on 1,000 liver biopsies. *Obes Surg*. 2004; 14:952–8. [PubMed: 15329185]

46. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007; 45:846–54. [PubMed: 17393509]
47. Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med*. 2011; 43:617–49. [PubMed: 21039302]
48. Harrison SA, Oliver D, Arnold HL, et al. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut*. 2008; 57:1441–7. [PubMed: 18390575]
- *49. Lee TH, Han SH, Yang JD, et al. Prediction of Advanced Fibrosis in Nonalcoholic Fatty Liver Disease: An Enhanced Model of BARD Score. *Gut Liver*. 2013; 7:323–8. This study identified an enhance model of the BARD score which can help predict advanced fibrosis. [PubMed: 23710314]
50. Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol*. 2012; 12:2. [PubMed: 22221544]
51. Cao W, Zhao C, Shen C, et al. Cytokeratin 18, alanine aminotransferase, platelets and triglycerides predict the presence of nonalcoholic steatohepatitis. *PLoS One*. 2013; 8:e82092. [PubMed: 24324749]
52. Wong GL. Update of liver fibrosis and steatosis with transient elastography (Fibroscan). *Gastroenterol Rep (Oxf)*. 2013; 1:19–26. [PubMed: 24759663]
53. Nobili V, Vizzutti F, Arena U, et al. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology*. 2008; 48:442–8. [PubMed: 18563842]
54. Lupsor M, Badea R, Stefanescu H, et al. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *J Gastrointest Liver Dis*. 2010; 19:53–60. [PubMed: 20361076]
- **55. Palmeri ML, Wang MH, Rouze NC, et al. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol*. 2011; 55:666–72. This was a novel study on the utility of acoustic radiation force impulse imaging in biopsy-proven NAFLD. [PubMed: 21256907]
56. Bota S, Herkner H, Sporea I, et al. Meta-analysis: ARFI elastography versus transient elastography for the evaluation of liver fibrosis. *Liver Int*. 2013; 33:1138–47. [PubMed: 23859217]
57. Friedrich-Rust M, Romen D, Vermehren J, et al. Acoustic radiation force impulse-imaging and transient elastography for non-invasive assessment of liver fibrosis and steatosis in NAFLD. *Eur J Radiol*. 2012; 81:e325–31. [PubMed: 22119555]
58. Osaki A, Kubota T, Suda T, et al. Shear wave velocity is a useful marker for managing nonalcoholic steatohepatitis. *World J Gastroenterol*. 2010; 16:2918–25. [PubMed: 20556839]
59. Ferraioli G, Tinelli C, Dal Bello B, et al. Accuracy of real-time shear wave elastography for assessing liver fibrosis in chronic hepatitis C: a pilot study. *Hepatology*. 2012; 56:2125–33. [PubMed: 22767302]
60. Poynard T, Munteanu M, Luckina E, et al. Liver fibrosis evaluation using real-time shear wave elastography: applicability and diagnostic performance using methods without a gold standard. *J Hepatol*. 2013; 58:928–35. [PubMed: 23321316]
61. Deffieux T, Gennisson JL, Bousquet L, et al. Investigating liver stiffness and viscosity for fibrosis, steatosis and activity staging using Shear Wave Elastography. *J Hepatol*. 2014
- **62. Cassinotto C, Lapuyade B, Mouries A, et al. Non-invasive assessment of liver fibrosis with impulse elastography: comparison of Supersonic Shear Imaging with ARFI and FibroScan®. *J Hepatol*. 2014; 61:550–7. This was a novel comparison study between acoustic radiation force impulse imaging and FibroScan®. [PubMed: 24815876]
63. Tutar O, Be er Ö, Adaletli I, et al. Shear wave elastography in the evaluation of liver fibrosis in children. *J Pediatr Gastroenterol Nutr*. 2014; 58:750–5. [PubMed: 24552673]
64. Lup or-Platon M, Stef nescu H, Mure an D, et al. Noninvasive assessment of liver steatosis using ultrasound methods. *Med Ultrason*. 2014; 16:236–45. [PubMed: 25110765]

65. Yoshioka K, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res.* 2014
66. Venkatesh SK, Ehman RL. Magnetic resonance elastography of liver. *Magn Reson Imaging Clin N Am.* 2014; 22:433–46. [PubMed: 25086938]
67. Huwart L, Sempoux C, Salameh N, et al. Liver fibrosis: noninvasive assessment with MR elastography versus aspartate aminotransferase-to-platelet ratio index. *Radiology.* 2007; 245:458–66. [PubMed: 17940304]
68. Huwart L, Sempoux C, Vicaute E, et al. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology.* 2008; 135:32–40. [PubMed: 18471441]
69. Yin M, Talwalkar JA, Glaser KJ, et al. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol.* 2007; 5:1207–1213. e2. [PubMed: 17916548]
- **70. Loomba R, Wolfson T, Ang B, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: A prospective study. *Hepatology.* 2014 This was the first prospective study of biopsy-proven NAFLD paired with MRI and MRE imaging modalities.
- **71. Chen J, Talwalkar JA, Yin M, et al. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology.* 2011; 259:749–56. This study helped develop the role of MRE in the detection of NASH. [PubMed: 21460032]
72. Loomba R, Sirlin CB, Ang B, et al. Ezetimibe for the treatment of nonalcoholic steatohepatitis: Assessment by novel MRI and MRE in a randomized trial (MOZART Trial). *Hepatology.* 2014
- **73. Lin SC, Heba E, Wolfson T, et al. Noninvasive Diagnosis of Nonalcoholic Fatty Liver Disease and Quantification of Liver Fat Using a New Quantitative Ultrasound Technique. *Clin Gastroenterol Hepatol.* 2014 This study identified a novel quantitative imaging biomarker, the quantitative ultrasound, for the diagnosis of NAFLD.
74. Yu LF, Zhong J, Cheng SD, et al. Low-dose azathioprine effectively improves mucosal healing in Chinese patients with small bowel Crohn's disease. *J Dig Dis.* 2014; 15:180–7. [PubMed: 24387287]
75. Verdam FJ, Dallinga JW, Driessen A, et al. Non-alcoholic steatohepatitis: a non-invasive diagnosis by analysis of exhaled breath. *J Hepatol.* 2013; 58:543–8. [PubMed: 23142062]
76. Banasch M, Ellrichmann M, Tannapfel A, et al. The non-invasive (13)C-methionine breath test detects hepatic mitochondrial dysfunction as a marker of disease activity in non-alcoholic steatohepatitis. *Eur J Med Res.* 2011; 16:258–64. [PubMed: 21810560]
77. Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology.* 2010; 52:904–12. [PubMed: 20648472]
78. Rotman Y, Koh C, Zmuda JM, et al. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology.* 2010; 52:894–903. [PubMed: 20684021]
79. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2008; 40:1461–5. [PubMed: 18820647]
80. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol.* 2014; 61:75–81. [PubMed: 24607626]
81. Singal AG, Manjunath H, Yopp AC, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol.* 2014; 109:325–34. [PubMed: 24445574]
82. Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med.* 2010; 362:1082–9. [PubMed: 20335584]
83. Verrijken A, Beckers S, Francque S, et al. A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. *Obesity (Silver Spring).* 2013; 21:2138–45. [PubMed: 23512881]
84. Sentinelli F, Romeo S, Maglio C, et al. Lack of effect of apolipoprotein C3 polymorphisms on indices of liver steatosis, lipid profile and insulin resistance in obese Southern Europeans. *Lipids Health Dis.* 2011; 10:93. [PubMed: 21663607]

85. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2014; 46:352–6. [PubMed: 24531328]
- *86. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology.* 2015; 61:506–14. This is a novel study that found a variant of TM6SF2 to be associated with cardiovascular disease risk. [PubMed: 25251399]
87. Hernaez R. Genetic factors associated with the presence and progression of nonalcoholic fatty liver disease: a narrative review. *Gastroenterol Hepatol.* 2012; 35:32–41. [PubMed: 22093607]
88. Mehta R, Biredinc A, Younossi ZM. Host genetic variants in obesity-related nonalcoholic fatty liver disease. *Clin Liver Dis.* 2014; 18:249–67. [PubMed: 24274878]
89. Ulitsky A, Ananthakrishnan AN, Komorowski R, et al. A noninvasive clinical scoring model predicts risk of nonalcoholic steatohepatitis in morbidly obese patients. *Obes Surg.* 2010; 20:685–91. [PubMed: 20336392]
90. Sumida Y, Yoneda M, Hyogo H, et al. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol.* 2011; 46:257–68. [PubMed: 20842510]
91. Pearce SG, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. *Biomark Res.* 2013; 1:7. [PubMed: 24252302]
92. Younossi ZM, Jarrar M, Nugent C, et al. A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obes Surg.* 2008; 18:1430–7. [PubMed: 18500507]
93. Palekar NA, Naus R, Larson SP, et al. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int.* 2006; 26:151–6. [PubMed: 16448452]
94. Chowdhury SD, Ramakrishna B, Eapen CE, et al. Fibrosis in non-alcoholic fatty liver disease: correlation with simple blood indices and association with tumor necrosis factor- α polymorphisms. *Trop Gastroenterol.* 2013; 34:31–5. [PubMed: 23923372]
95. McPherson S, Stewart SF, Henderson E, et al. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut.* 2010; 59:1265–9. [PubMed: 20801772]
96. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology.* 1988; 95:734–9. [PubMed: 3135226]
97. Poynard T, Ratziu V, Naveau S, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol.* 2005; 4:10. [PubMed: 16375767]
98. Loaeza-del-Castillo A, Paz-Pineda F, Oviedo-Cárdenas E, et al. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. *Ann Hepatol.* 2008; 7:350–7. [PubMed: 19034235]
99. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology.* 2008; 47:455–60. [PubMed: 18038452]
100. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology.* 2004; 127:1704–13. [PubMed: 15578508]
101. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2009; 7:1104–12. [PubMed: 19523535]
102. Tahan V, Canbakan B, Balci H, et al. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. *Hepatogastroenterology.* 2008; 55:1433–8. [PubMed: 18795706]
103. Calès P, Boursier J, Chaigneau J, et al. Diagnosis of different liver fibrosis characteristics by blood tests in non-alcoholic fatty liver disease. *Liver Int.* 2010; 30:1346–54. [PubMed: 20666992]
104. Alkhouri N, Mansoor S, Giammaria P, et al. The development of the pediatric NAFLD fibrosis score (PNFS) to predict the presence of advanced fibrosis in children with nonalcoholic fatty liver disease. *PLoS One.* 2014; 9:e104558. [PubMed: 25121514]

Table 1a

Clinical predictors and biomarkers for NASH

Test name or study	Markers	Purpose of test	AUROC training	AUROC validation
Ulitsky et al. ⁸⁹	DM, TG, ALT, OSA	Distinguishing NASH from NAFLD in morbidly obese individuals	0.76	Not reported
NAFIC ⁹⁰	Fasting insulin, type 4 collagen 7S, ferritin	Distinguishing NASH from NAFLD	0.85	0.78
NASH clinical scoring system ⁹¹	Hypertension, DM type 2, sleep apnea, nonblack race, AST>27IU/L, ALT>27IU/L	Distinguishing NASH from NAFLD in bariatric surgery patients	0.80	0.75
OxNASH ³⁶	BMI, age, AST, 13- HODE	Distinguishing NASH from NAFLD	0.83	0.74
MeBT ⁷⁶	(13)C-methionine	Distinguishing NASH from NAFLD	0.87	Not reported
Younossi et al. ⁹²	Resistin, CK-18, cleaved CK-18, adiponectin	Distinguishing NASH from NAFLD	0.91	0.73
Tamimi et al. ²¹	CK-18, sFas	Distinguishing NASH from NAFLD	0.93	0.79
Machado et al. ²⁸	Adiponectin, leptin, ghrelin	Distinguishing NASH from NAFLD in morbidly obese individuals	0.79	Not reported
Tanwar et al. ²⁴	Terminal peptide of procollagen III	Distinguishing NASH from NAFLD	0.83	0.78
Cao et al. ⁵¹	CK-18, ALT, platelets, TG	Distinguishing NASH from NAFLD	0.92	Not reported
Verdam et al. ⁷⁵	n-tridecane, 3- methyl- butanonitrile, 1- propanol	Distinguishing NASH from NAFLD in bariatric surgery patients	0.77	Not reported
Palekar et al. ⁹³	Age, sex, AST, BMI, AST/ALT ratio, serum HA	Distinguishing NASH from NAFLD	0.76	Not reported
HAIR ⁴⁴	Hypertension, ALT, insulin resistance	Distinguishing NASH from NAFLD in bariatric surgery patients	0.90	Not reported

Abbreviations: BMI: body mass index; NASH: nonalcoholic steatohepatitis; AUROC: area under receiver operating characteristic; DM: diabetes mellitus; AST: aspartate aminotransferase; 13-HODE: 13-(S)-hydroxyoctadecadienoic acid; INR: international normalized ratio; TG: triglycerides; OSA: obstructive sleep apnea; PT: prothrombin time; CK-18: cytokeratin 18; sFas: soluble Fas ligand; HA: hyaluronic acid; ALT: alanine aminotransferase; NAFIC: Ferritin, fasting insulin, type 4 collagen 7S; MeBT: (13)C-methionine; HAIR: hypertension, ALT, insulin resistance

Table 1b

Clinical predictors and biomarkers for advanced fibrosis

Test name or study	Year created	Markers	AUROC training	AUROC validation
AST/ALT ⁹⁴⁻⁹⁶	1988	AST, ALT	0.83	0.83–0.90
BAAT ⁴⁰	2000	BMI, ALT, TG	0.84	Not reported
Fibro test ⁹⁷	2001	ALT, haptoglobin, alpha- macroglobulin, GGT, apolipoprotein A1, total bilirubin	0.86	0.75
APRI ^{94, 95, 98}	2003	AST, platelets	0.80	0.57–0.68
ELF score ^{99, 100}	2004	HA, TIMP1, P3NP	0.87	0.90
Fibrometer ⁹⁹	2005	Glucose, AST, weight, ferritin, ALT, age, platelets	0.94	0.94
FIB-4 ^{95, 101}	2006	Age, AST, ALT, platelets	0.80	0.86
NAFLD fibrosis score ^{46, 95, 101}	2007	Age, albumin, AST/ALT ratio, hyperglycemia, platelet count, BMI	0.88	0.77–0.84
BARD ^{48, 95}	2008	BMI, DM, AST, ALT	0.81	0.77–0.78
GGT ¹⁰²	2008	GGT	0.74	Not reported
Modified ELF ⁹⁹	2008	ELF score, BMI, platelets, DM, albumin, AST, ALT	0.98	Not reported
Cales et al. ¹⁰³	2010	Weight, age, AST, ALT, PT	0.87	Not reported
BARDI ⁴⁹	2013	BMI, DM, AST, ALT, INR	0.88	Not reported
PNFS ¹⁰⁴	2014	ALT, AP, platelets, GGT	0.74	Not reported

Abbreviations: BMI: body mass index; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; DM: diabetes mellitus; ALT: alanine aminotransferase; TG: triglycerides; PT: prothrombin time; CK-18: cytokeratin 18; GGT: gamma-glutamyl transpeptidase; AP: alkaline phosphatase; HA: hyaluronic acid; TIMP-1: tissue inhibitor of metalloproteinases-1; P3NP: procollagen type III N-terminal peptide; AST: aspartate aminotransferase; AUROC: area under receiver operating characteristic; INR: international normalized ratio; BARDI: BMI, DM, AST, ALT, INR; OxNASH: BMI, age, AST, 13-HODE; NAFIC: Ferritin, fasting insulin, type IV collagen 7S; ELF: European liver fibrosis score; BARD: BMI, AST/ALT ratio, diabetes; BAAT: BMI, ALT, TG; FIB-4: fibrosis index with 4 components: age (year) x AST (U/L)/platelet count (10 /L) x (ALT(U/L))(1/2); APRI: AST platelet ratio index; PNFS: Pediatric NAFLD fibrosis score

Table 2

Imaging tools for NASH and advanced fibrosis

Test name	Main function of test	Pros	Cons
US	Identifying steatosis, ruling out cirrhosis	Readily available, inexpensive, easy to perform, fast	Not sensitive in fatty liver disease, qualitative measure of fat, not accurate in diagnosing NASH or advanced fibrosis, operator dependent
CT	Identifying steatosis, ruling out cirrhosis	Readily available, easy to perform, fast	Not sensitive in fatty liver disease, qualitative measure of fat, not accurate in diagnosing NASH or advanced fibrosis, exposure to radiation
TE	Identifying and staging fibrosis	Fast, inexpensive, easy to perform, accurate in predicting fibrosis, has standardized procedure	Poor at identifying NASH without fibrosis, high failure rate, specialized probes needed for pediatric and obese and ascites, operator dependent, measures in one dimension
ARFI	Identifying and staging fibrosis	Fast, accurate, uses conventional US probe so one machine for full US evaluation of liver including specific areas of interest, superior in obese/ascites than TE	Poor at identifying NASH without fibrosis, no standardized procedure, operator dependent
SWE	Identifying and staging fibrosis	Fast, accurate, produces real-time imaging, superior in obese/ascites than TE	Poor at identifying NASH, no standardized procedure
MRE	Identifying and staging fibrosis, identifying NASH in patients without fibrosis	Most accurate noninvasive method, low failure rate, can be used in pediatric, ascites, or obese patients, no contrast or radiation, potential to identify NASH without fibrosis	Expensive, expertise needed to analyze, not readily available, less validated than biopsy or TE

Abbreviations: NASH: nonalcoholic steatohepatitis; TE: transient elastography; CT: computed tomography; ARFI: acoustic radiation force impulse; MRE: magnetic resonance elastography; SWE: shear wave elastography; US: ultrasound