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Permalink

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Publication Date

2020-10-01

DOI

10.1016/j.metabol.2020.154259

Peer reviewed

Advances in non-invasive biomarkers for the diagnosis and monitoring of non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most common chronic liver disease in the United States, affecting approximately 1 out of every 4 Americans. NAFLD is a spectrum of disorders including simple steatosis, characterized by the presence of hepatic steatosis with minimal inflammation, and nonalcoholic steatohepatitis (NASH), characterized by the presence of hepatic steatosis with lobular inflammation, ballooning with or without peri-sinusoidal fibrosis. NASH may lead to progressive fibrosis, and therefore, Individuals with NASH and, in particular, hepatic fibrosis are at increased risk for both liver- and cardiovascular-related outcomes compared

to those with steatosis alone. New treatments for NASH and hepatic fibrosis are emerging, so now, more than ever, it is important to identify individuals with more advanced disease who may be candidates for therapy. Noninvasive methods to accurately diagnosis, risk stratify, and monitor both NASH and fibrosis are critically needed. Moreover, since clinically relevant outcomes, such as developing end stage liver disease or liver cancer, take many years to develop, reliable surrogate markers of outcome measures are needed to identify and evaluate potential therapies. In this review, we discuss methods to noninvasively diagnosis and monitor both NASH and fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is now the most common chronic liver disease in western societies.[1, 2] NAFLD is characterized by increased fatty infiltration of the liver, in the absence of secondary causes. Most patients with NAFLD have fatty infiltration of the liver without significant inflammation, so called simple steatosis, whereas approximately 20% of individuals with NAFLD have non-alcoholic steatohepatitis (NASH), which is marked by lobular inflammation, ballooning of hepatocytes and fibrosis formation.[3] Individuals with NASH can develop progressive fibrosis, which ultimately leads to end stage liver disease manifesting as cirrhosis, hepatocellular carcinoma, and increased risk for liver-related mortality.[4, 5] In addition to liver-related outcomes, individuals with NAFLD, particularly those with NASH and fibrosis, are at increased risk for cardiovascular disease, cancer, diabetes and all-cause mortality.[6–9]

Recently, it was noted that even early stages of hepatic fibrosis increase the risk for liver-related complications and death.[5] In order to prevent liver-related death, it is important to diagnosis individuals with NASH and fibrosis, when the disease is still reversible through lifestyle modification and, hopefully, through emerging therapies. For inclusion in clinical trials for NASH, most studies require biopsy confirmed NASH with a fibrosis stage of at 2 or more. Improvement and/or reversal of NASH is considered a major target of clinical trials for novel therapeutic agents aimed at lowering the burden of liver disease secondary to NAFLD.[10] Advanced liver fibrosis, defined by NASH clinical research network (CRN) criteria with stage 3 defined as bridging fibrosis and stage 4 as cirrhosis, is the most powerful predictor of adverse liver- and cardiovascular-related outcomes and is also considered a major target for therapeutic trials.[10] Therefore, NASH and fibrosis are diagnostic imperatives and the development of biomarkers to accurately diagnose and monitor NASH activity and fibrosis progress/regression are critically important.

There are many unanswered questions in the natural history of NAFLD and NASH, largely because of the challenges in diagnosis and monitoring of disease progression. The gold standard for diagnosing and monitoring NAFLD is liver biopsy.[3] However, liver biopsy is costly and invasive and, in clinical practice, it is not possible or appropriate to biopsy all patients with suspected NAFLD.[11] Though liver biopsy is considered safe, there remains a risk of serious complication, including, pain at the biopsy site, serious bleeding, and, rarely, death. Liver biopsy is also limited by variability in tissue sampling and interobserver and intraobserver variability in interpretation.[12] In this review, we will discuss both established and emerging non-invasive tests (NITs) for NASH and fibrosis. These biomarkers may be

useful to diagnosis disease, select patients for treatment, predicting outcomes and monitor disease activity.

Diagnosis, risk stratification and patient selection

NAFLD is routinely identified on standard imaging techniques, such as ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). Whereas the majority of those with NAFLD has simple steatosis, it is not possible to distinguish NASH from simple steatosis using imaging alone. Additionally, hepatic fibrosis, particularly if portal hypertension is not well established, is not distinguished on standard imaging techniques. A number of blood-and imaging-based NITs have been developed with great success to assist in the noninvasive diagnosis of hepatic fibrosis, though NITs for NASH are still critically needed.

Noninvasive prediction of steatohepatitis: blood-based biomarkers—NASH is a complex process that involves a number of disturbances including increased hepatocellular apoptosis, inflammation, oxidative stress, and altered adipokine signaling. Prior investigations have focused on identifying individual biomarkers which may reflect alterations in cellular and molecular processes in NASH. Serum aminotransferase levels, most commonly alanine aminotransferase (ALT), are often used to identify individuals with possible NASH; however, both the sensitivity and specificity for NASH are relatively low (sensitivity 64% and specificity 75%).[13] In a study of biopsy-confirmed NASH, over one third of participants a with normal ALT (< 35 Units/Liter) had NASH or advanced fibrosis and more than half of those with elevated ALT (35 Units/Liter) did not have NASH or advanced fibrosis.[14, 15] The area under the receiver operating characteristic curve (AUROC) of ALT for detecting NASH is consistently poor (0.61–0.62), in multiple studies.[14, 15]

Apoptosis and resultant hepatocyte death and release of cytokeratin 18 (CK18) fragments is increased in NASH compared to simple steatosis. Different assays, measuring the two types of CK18 fragments, have been extensively studied as potential biomarkers to distinguish active NASH from simple steatosis. A pooled meta-analysis of 11 studies and over 800 patients showed that CK18 had a pooled sensitivity of 66% and specificity of 82%.[16] Using optimal cut-offs can improve sensitivity to 82% and specificity to 98%; however, there was considerable variability in how optimal cut-offs were defined between studies.[16] Cut-off values of CK-18 ranged widely – from 111.6–380.0 Units/Liter when maximizing sensitivity and from 261.4–670 Units/Liter when maximizing specificity.[16] Additional studies are needed to validate optimal CK-18 cut-offs in various populations and to determine if CK18 alone or in combination with other measures helps identify patients with likely NASH better than ALT.

Since inflammation is a hallmark feature of NASH, various inflammatory markers have been studied as potential NASH biomarkers. In the NASH Clinical Research Network, activated plasminogen activator inhibitor 1 (PAI-1) was associated with histologic NASH compared to non-NASH samples (OR 1.2, 95% confidence interval (CI) 1.08–1.34, p < 0.001).[17] PAI-1 is a regulator of fibrinolysis and may contribute to both liver and vascular fibrosis.[18] In another study, elevation in PAI-1 was associated with a positive relationship between NASH and

fibrosis, even after adjustment for metabolic syndrome and insulin resistance.[19] Additional biomarkers including interleukin-8, monocyte chemoattractant protein-1, resistin, soluble interleukin-1 receptor I, soluble interleukin-2 receptor alpha, tumor necrosis factor alpha associated with histologic fibrosis, but not NASH after accounting for clinical and metabolic factors.[17] Markers of oxidative stress have also been tested as potential biomarkers for NASH. The oxNASH panel, which combines simple clinical and laboratory values with the ratio of 13-hydroxy octadecadenoic acid to linoleic acid (oxNASH), predicted NASH better than AST or ALT alone.[20] Using low and high cutoff thresholds, oxNASH had a sensitivity of 81% to exclude NASH (oxNASH below 55) and a specificity to detect NASH of 97% (oxNASH above 73), though additional validation studies are needed.[20] Hepatic inflammation also contributes to lipotoxicity, which also may play an important role in the progression to NASH and fibrosis.[21] Novel techniques using liquid chromatography/mass spectroscopy to perform lipidomic profiling of individuals with NASH compared to simple steatosis can be utilized to identify novel biomarkers.[22–24] In a recent proof-of-concept study, we observed that a single eicosanoid biomarker was able to distinguish NASH from simple steatosis with an AUROC of 1.[22] However, confirmatory studies are needed before lipidomic biomarkers can be used in clinical practice.

Fibroblast growth factor 21 (FGF21) is a hormone-like growth factor expressed in liver, adipose, and pancreas tissue, which is involved in many metabolic processes including insulin sensitivity and lipid metabolism.[25] FGF21 also impacts other metabolically-active hormones, including adiponectin and leptin.[25] Chronic exposure to FGF21 increases adiponectin levels in humans and a pegylated FGF21 analogue is currently in clinical trials as a potential treatment for NASH.[26] One study evaluated the role of FGF21 as a biomarker for NASH compared or combined with CK18.[27] Combining FGF21 and CK18 demonstrated better accuracy for NASH compared to either biomarker alone with an area under the receiver operator characteristic (AUROC) curve of 0.94 (95% CI 0.92–0.96).[27] Another study tested a stepwise approach starting with CK18 followed by FGF21 testing in those with high or indeterminate CK18 fragments.[28] This study also observed a high sensitivity and specificity for NASH; however, the majority of patients (>70%) were in the indeterminate range or had discrepant results, which limited the clinical usefulness.[28] Additional studies that account for comorbid metabolic conditions are needed to validate these findings. Moreover, FGF21 is regulated by genes that display circadian regulation[29] and FGF21 levels follow a circadian rhythm with levels fluctuating throughout the day.[30] Future studies should examine the potential effects of the circadian cycle and relationships to the fed/fasting state on levels of FGF21 or other possible metabolic biomarkers for NASH. Neither FGF21 nor CK18 are available for clinical use and more studies are needed before they can be widely recommended.

Noninvasive prediction of fibrosis- blood-based biomarkers—In contrast to NASH biomarkers, multiple biomarkers and clinical prediction models for hepatic fibrosis exist and have been extensively studied and validated as discussed in detail in a recent review paper.[31] The NAFLD Fibrosis Score (NFS)[32] and Fibrosis-4 index[33] (FIB4) are blood-based diagnostic models for hepatic fibrosis with high negative predictive values to exclude advanced fibrosis.[34] However, approximately 30% of participants will fall in the

indeterminate range of these models and both tests are limited for the diagnosis of earlier stages of fibrosis.[35] The FIB4 index incorporates age, ALT, aspartate aminotransferase (AST), and the platelet count into a simple formula, which was originally developed for hepatitis C and HIV co-infection.[33] The NFS was specifically developed for NAFLD and it uses age, body mass index (BMI), the presence of diabetes or impaired fasting glucose, AST:ALT ratio, platelet count and serum albumin in a simple online calculator (www.nafldscore.com) to predict the risk for advanced fibrosis.[32] The NFS performs well at excluding advanced fibrosis for those with a value below the lower cutoff of −1.455 with a negative predictive value of 88%.[32] At the higher cutoff (0.676), the positive predictive value for advanced fibrosis was 82%.[32] In a recent meta-analysis, both the NFS and FIB4 had similar accuracy for detecting advanced fibrosis in NAFLD.[34] It is important to recognize the limitations of the NFS and FIB4 tests. The NFS and FIB4 were both derived in hospital-based samples composed of patients who had undergone liver biopsy. For example, caution is needed when applying these NITs to the general population since the scores may be miss calibrated to detect advanced fibrosis in a population with a much lower disease prevalence. [36] Moreover, since both NFS and FIB4 were derived from samples of middle-aged participants, different cutoffs may be needed among young participants (< 35 years old) and older participants (65 years old) to avoid under-diagnosis and over-diagnosis of suspected fibrosis, respectively.[37]

Noninvasive prediction of steatohepatitis: imaging-based biomarkers—
Imaging-based biomarkers for NASH have been evaluated in numerous studies; however, neither ultrasound- or MRI-based technologies are consistently accurate at identifying NASH.[38,] As many individuals with NASH also have fibrosis, it is not known if elastography-based imaging techniques are measuring NASH alone or fibrosis. Large, well-phenotyped datasets of participants with NASH without fibrosis are lacking, which has limited the development of imaging biomarkers for NASH. The FibroScan-AST (FAST) score is an imaging-based biomarker panel which includes AST along with vibration-controlled elastography-derived values of fat (the controlled attenuation parameter (CAP) and the liver stiffness measurement (LSM) see next section), recently showed promise as a biomarker for "active NASH".[40] The FAST score was validated in several cohorts with varying prevalence of NAFLD; however, the positive predictive value ranged from 0.33 to 0.83 depending the cohort. Additional real-world validation studies are needed as are studies evaluating the association between FAST and response to treatment.

Noninvasive prediction of fibrosis: imaging-based biomarkers—Imaging modalities for advanced fibrosis can be divided into ultrasound- and magnetic imaging resonance (MRI)-based. The most frequently utilized ultrasound-based technology is vibration-controlled transient elastography (VCTE; FibroScan, Paris, France), which was approved by the Food and Drug Administration (FDA) in 2015. VCTE is often used in the hepatology clinic to provide point-of-care evaluation of hepatic fibrosis. VCTE uses a modified ultrasound probe that is placed in the intercostal space and delivers a 50 MHz shear wave that propagates through the liver tissue. VCTE simultaneously estimates the liver stiffness, measured in KiloPascals (kPa), which correlates with hepatic fibrosis, and the controlled attenuation parameter (CAP), measured in Decibels/meter (Db/m), which

correlates with steatosis. Multiple probe sizes are available including: the M probe (3.5 MHz), generally used in adults, the XL probe (2.5 MHz), designed for patients with central obesity, and the S probe (5 MHz), which is designed for use in children. To be considered valid, a trained operator obtains a minimum of 10 images per VCTE exam and the device automatically calculates the median LSM and CAP and the ratio of the interquartile range to median (IQR/M) LSM value. Exam quality is measured in two ways. First, all images obtained should be reviewed to ensure they are of high quality. High quality examinations should also be consistent, which is evaluated by the IQR/M value. A scan is 'very reliable' if the IQR/M ≤ 0.10, 'reliable' if the IQR/M is greater than 0.1 or less than or equal to 0.3. If the IQR/M is greater than 0.3 the scan is still considered 'reliable' if the median LSM is less than 7.1 kPa, but if the LSM is greater than or equal to 7.1 KPa then the scan is considered 'poorly reliable'.[41] A wide range of LSM cut-off values for advanced fibrosis have been evaluated in the literature (ranging from 5.9 kPa to 12kPa) and there is considerable variability between studies.[34, 42–44] Additionally, the cutoff values to diagnosis advanced fibrosis may be lower on the XL probe compared to the M probe.[45] In a study of approximately 400 patients with biopsy-proven NAFLD, a LSM cut-off of 8.6 kPa provided the best balance between sensitivity and specificity for ruling in at least moderate liver fibrosis (F ≥ 2)[43] (Table 1). VCTE performs best at ruling out advanced fibrosis with a consistently high negative predictive value between studies.[38] In addition to fibrosis, a number of other factors may elevate the LSM including right heart failure, inexperienced operator, hepatic inflammation with high ALT, recent food ingestion, cholestasis, and active alcohol use.[46] These factors should be considered when interpreting VCTE results, particularly in the setting of an unexpectedly high LSM.

Other ultrasound-based technologies include supersonic shear imaging (SSI)[47] and acoustic radiation force impulse (ARFI)[48], which are both integrated into a standard ultrasound unit. For SSI, focused ultrasonic beams create the shear wave and a very high frame rate ultrasound sequence captures the real time propagation of the shear wave through the liver.[49] The shear wave speed is measured by a Doppler-like acquisition over a 1 cm region of interest that is selected by the operator and the device automatically calculates the mean liver stiffness over the region of interest. A fibrotic liver will be stiffer and appear red. The screen displays the mean, minimum, maximum and standard deviation of the measurement, as well as the diameter of the circle. For ARFI, an acoustic 'push' generates the shear wave over the selected region of interest. The resultant shear wave is tracked as it propagates through the liver tissue by the ultrasound receiver, which estimates the shear-wave speed in meters per second, which is proportional to the square root of the shear moduli (measured in kPa). The operator determines if the images are adequate after evaluating for motion artifact or other issues with image acquisition. A study found that SSI, ARFI, and VCTE all had similarly high performance for the evaluation of advanced fibrosis compared to liver biopsy (AUROC for F ≥ 3 fibrosis stage: 0.89, 0.84, 0.86, respectively).[50] Obesity contributed to study failure or unreliable results for SSI, ARFI, and VCTE, though this study was conducted before the VCTE XL probe was available.[50]

Magnetic Resonance Elastography (MRE) is a reproducible method to non-invasively measure hepatic fibrosis by imaging approximately 5% of the liver.[51] MRE uses specialized hardware and software added to a conventional MRI. A circular device placed anterior to the

liver is attached to the patient and connected to an active acoustic driver located outside of the MRI room. As the patient perform breath holds, the device generates shear waves at 60 Hz which propagate through the liver. Images are interpreted using a commercial software to generate multicolor maps of liver stiffness over specified regions of interest. Unlikely ultrasound-based elastography, MRE can discriminate between different hepatic fibrosis stages.[34, 38, 52] Additionally, MRE consistently is the most accurate elastography method (AUROC 0.93) at a cut off of 3.6 kPa (Table 1).[34, 38] MRE failure rate is low, though increased hepatic iron, inflammation, or right heart failure may result in failure or false positive results.[53, 54] MRE may also not be possible in patients with implanted metallic devices or who exceed the weight limitations of the MRI scanner. In head to head comparative studies in patients with biopsy-proven NAFLD with contemporaneous MRE and VCTE, MRE is consistently superior than VCTE in differentiating each stage of fibrosis.[38, 39, 55] A comparison of the clinical use and advantages and limitations of various NITs for NAFLD (including NASH and hepatic fibrosis) is presented in Table 2.

Novel biomarkers: The human intestinal microbiota may contribute to the progression of simple steatosis to NASH and fibrosis though influencing hepatic lipid and bile acid metabolism and contributing to endogenous alcohol consumption.[56] Studies of the microbiome composition may be useful as a non-invasive method to distinguish between various NAFLD phenotypes. In a small cohort of patients with NAFLD at various stages, disease severity was associated with distinct microbiota signatures with an increase in *Bacteroides* in those with NASH compared to non-NASH and an increase in *Ruminococcus* in those with stage 2–4 fibrosis compare to those without significant fibrosis.[57] Though another study, which evaluated bacterial DNA from blood-based samples, found lower *Ruminococcaceae* in patients with hepatic fibrosis.[58] In a cohort of biopsy-defined NAFLD, a gut microbiome-derived metagenomic signature was highly accurate in detecting the presence of advanced fibrosis (AUROC 0.936).[59] Using an integrative multi-omics approach in a multi-center cohort of well-phenotyped, non-obese women without diabetes, Hoyles et al identified cross-talk between the gut microbiome and host gene expression and metabolism.[60] Individuals with hepatic steatosis had low microbial gene richness and a distinct molecular signature that robustly distinguished individuals with hepatic steatosis with an AUROC of 0.87.[60] They noted that aromatic amino acids are associated with NAFLD, and these findings were also replicated in a twin-family based study, comparing those with advanced fibrosis based upon MRE versus those with those with no or mild/ moderate fibrosis.[61] Utilizing a family-based study including probands with NAFLD cirrhosis and referrants and their respective first-degree relatives, a gut microbiome signature for NAFLD cirrhosis was developed using 27 bacterial features and it was able to detect advanced fibrosis among first-degree relatives of these probands.[62] These data suggest that gut microbiome derived signature may be used as a diagnostic test for advanced fibrosis or cirrhosis in patients with NAFLD. Additional human studies are needed to determine if microbiobial signatures are generalizable across different populations and remain after adjusting for confounding factors.

Advances in 'omics' research over the last decade has led to important insights into the pathophysiology of NAFLD. Biomarkers derived from metabolomics may be useful in

differentiating NAFLD phenotypes. One small study found that a machine learning-derived model which included lipid, glycan, and hormonal biomarkers was highly accurate at distinguishing between NASH, simple steatosis and healthy phenotypes.[24] Additional studies are needed to identify additional biomarkers and for validation in other cohorts.

Biomarkers of disease activity and clinical outcomes

In addition to diagnosis and risk stratification, it is critically important to identify biomarkers that accurately measure changes in NASH and hepatic fibrosis in order to monitor disease progression and response to therapy. As many of the clinically important outcomes in liver disease, such as progression to cirrhosis, decompensation, and death, thankfully, take many years to develop, surrogate markers, which are predictive of future events are also needed.

Blood-based biomarkers of disease activity—The simplest biomarker of treatment response is ALT, which has been utilized as a surrogate marker of liver damage in several studies. In the treatment of nonalcoholic fatty liver disease in children (TONIC) study, each unit decrease in ALT was associated with a 30% increased odds of histologic improvement[63] and mean change in ALT is considered a proxy for histologic improvement in NASH.[64]

Few studies have evaluated the ability of blood-based fibrosis models to detect changes in histologic fibrosis. In a study of 261 patients with biopsy-confirmed NASH at baseline and 1 year after lifestyle intervention, changes in NFS were independently associated with fibrosis improvement or progression.[65] Though change in platelet count, which is a component of the aspartate-aminotransferase-to-platelet ratio index (APRI) and FIB4 index, also associated with fibrosis improvement or progression, no associations between changes in APRI or FIB4 and fibrosis change were observed.[65] However, in a secondary analysis of the Phase 2B Farnesoid X Receptor Ligand Obeticholic Acid in Non-alcoholic Steatohepatitis Treatment (FLINT) trial, patients with histologic fibrosis improvement at week 24 demonstrated reductions in APRI, FIB4, and NFS; however, only reductions in APRI and FIB4 significantly correlated with at least a 1 stage improvement in fibrosis at week 72.[66] In a phase 2 clinical trial, improvement in the Enhanced Liver Fibrosis (ELF) test associated with fibrosis regression[67]; though in another secondary analysis, the ELF test did not associate with progression to cirrhosis.[68] A new model for predicting fibrosis improvement consisting of glycated hemoglobin, platelets, and ALT shows promise with an AUROC of 0.96 for fibrosis improvement, though external validation is needed before this model can be incorporated into practice.[65]

Imaging-based biomarkers of disease activity—The usefulness of ultrasound-based elastography modalities to monitor disease activity is not known as there may be substantial variability in measurements unrelated to regression or progression of fibrosis.[69] Longitudinal studies with serial VCTE examinations and histologic evaluations are limited so more data are needed. There is some evidence in chronic hepatitis B that longitudinal changes in VCTE-liver stiffness may relate to histologic progression of disease, but additional studies are needed.[70]

Magnetic resonance imaging-estimated protein density fat fraction (MRI-PDFF) is a quantitative biomarker of hepatic steatosis. High MRI-PDFF is also associated with early

fibrosis progression and less improvement in NASH.[71] Prior studies have demonstrated that MRI-PDFF correlates with histologic steatosis grade in both cross-sectional[72] and longitudinal studies.[73, 74] Improvement in MRI-PDFF is used to assess longitudinal changes in hepatic steatosis in clinical studies of NAFLD[67, 75–77] at early stages of development.[78] A reduction of MRI-PDFF by 30% is associated with histologic improvement in NAFLD Activity Score.[79] These data were subsequently validated in the Selonsertib Phase 2b trial as well as in a subset of the FLINT Trial[80], and now confirmed in a recent trial using Resmetirom versus placebo for the treatment of NASH.[67]

MRE has also been studied as a potential imaging modality to monitor disease activity. The majority of studies which have evaluated the performance of MRE compared to liver histology are cross-sectional and limited longitudinal data exist. In a secondary-analysis of patients enrolled in a phase 2 study of selonsertib, increases or decreases in MRE-liver stiffness significantly associated with histologic fibrosis progression or regression, respectively; though the sample size was small and the AUROCs were modest.[67] Recently, a small, well-characterized cohort of patients with NAFLD and paired liver histology and MRE measurements demonstrated that a 15% increase in MRE was associated with histologic fibrosis progression, even after accounting for baseline BMI.[81] However, a worsening of liver stiffness on MRE by 15% was associated with higher odds of histologic progression of fibrosis.[81] Additional studies are needed to assess the association between changes in MRE liver stiffness and clinically relevant liver outcomes – including progression to cirrhosis, decompensation and liver-related death.

Non-invasive biomarkers and clinical outcomes—In a retrospective cohort study, elevated APRI and FIB4 were associated with liver-related outcomes over up to 8 years of follow-up.[82] In a separate study, an ELF score ≥ 9.49 had a higher sensitivity and specificity for predicting liver-related outcomes over 6 years compared to liver biopsy.[83] Change in liver stiffness may be an important biomarker of liver related events, though specific studies in NAFLD are needed. In one study, LSM derived from VCTE had a similarly high discriminative ability to predict liver-related mortality compared to FIB4.[84] Additionally, in a longitudinal cohort, each stage increase in VCTE-defined fibrosis stage was associated with a lower overall survival.[84] In a meta-analysis, high liver stiffness was associated with a significant risk for hepatic decompensation (relative risk 1.07; 95% CI 1.03–1.11), liver cancer (RR 1.11; 95% CI, 1.05–1.18), and death (RR, 1.22; 95% CI, 1.05–1.43).[85] In a study of patients with primary biliary cirrhosis, chronic cholestatic liver disease, a 2.1 kPa per year increase in LSM derived from VCTE was associated with an 8-fold increased risk of liver decompensation, liver transplantations, and deaths.[86] Baseline liver stiffness measure by SSI correlated with hepatic venous pressures gradient.[87] Changes in liver stiffness, measured by SSI, were strongly correlated with changes in the hepatic venous pressure gradient measurements, a measurement of portal hypertension, indicating a possible role for SSI to predict clinically significant portal hypertension and response to therapy.[88]

Conclusions:

Accurate methods to diagnosis and monitor NASH and hepatic fibrosis are critically important for ongoing progress towards preventing liver-related morbidity and mortality.

Though there has been significant progress in the noninvasive diagnosis of hepatic fibrosis, advances in the noninvasive diagnosis of NASH lag behind. Large, diverse, cohorts of participants who are well-characterized for NAFLD, NASH, and fibrosis are needed for biomarker discovery. Genomics, metabolomics, and other "omics" technologies may be useful in improving our understanding of disease mechanisms and identifying novel biomarkers of disease activity. However, deep phenotyping and genotyping of large cohorts of participants are needed for both discovery and validation of potential biomarkers.

Despite advances, there still remains many unanswered questions in the non-invasive diagnosis of fibrosis, including a lack of clarity as to the optimal cutoff values for different imaging modalities and in different patient populations. NITs may be useful for risk stratification, which can lower costs and specialist referrals and reduce the number of patients subject to the risks of liver biopsy. However, best practices for using NITs to risk stratify or select patients for treatment are needed as there currently is a lot of practice variability. As new therapies are evaluated for NASH and fibrosis, we need to understand how changes in blood- and imaging-based biomarkers may relate to disease activity. Currently we rely on repeat liver biopsy to monitor disease activity, but this method is costly, invasive, and, ultimately, hindering medical progress. We need to invest in creating diverse cohorts of participants whom have undergone multiple non-invasive measures of fibrosis and who are monitored for the development of important clinical outcomes. Such cohorts will add in the development and validation of novel biomarkers that predict clinically relevant outcomes which are urgently needed as the prevalence of hepatic fibrosis secondary to NASH continues to rise

Grant Support/Acknowledgements

Abbreviations:

NAFLD	Non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NITs	noninvasive tests

CT	computed tomography
US	ultrasound
MRI	magnetic imaging resonance
ALT	alanine aminotransferase
CK18	cytokeratin 18
PAI-1	plasminogen activator inhibitor
OR	odds ratio
CI	confidence interval
AUROC	area under the receiver operator characteristic
oxNASH	13-hydroxy octadecadenoic acid to linoleic acid
FGF21	fibroblast growth factor 21
NFS	NAFLD fibrosis score
FIB4	fibrosis 4 index
BMI	body mass index
AST	aspartate aminotransferase
VCTE	vibration-controlled transient elastography
IQR/M	interquartile range/median
LSM	liver stiffness measurement
CAP	controlled attenuation paramete
SSI	supersonic shear imaging
ARFI	acoustic radiation force imaging
MRE	magnetic resonance elastography
MRI-PDFF	magnetic resonance imaging – proton density fat fraction
FDA	food and drug administration
FLINT	Farnesoid X Receptor Ligand Obeticholic Acid in Non-alcoholic Steatohepatitis Treatment
APRI	aspartate-to-platelet ratio index
ELF	the Enhanced Liver Fibrosis
TONIC	treatment of nonalcoholic fatty liver disease in children

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Highlights

- Non-alcoholic fatty liver disease is the most common chronic liver disease

- Simple steatosis cannot be distinguished from steatohepatitis non-invasively

- Biomarkers for steatohepatitis are being developed but are not available yet for clinical use

- Hepatic fibrosis can be identified non-invasively using imaging or blood based biomarkers

- Magnetic Resonance Elastography provides the most accurate method for non-invasively diagnosing hepatic fibrosis

Table 1:

Vibration-Controlled Transient Elastography and Magnetic Resonance Elastography Based-Assessment of Liver Fibrosis.

Stage	Modality	AUROC	Threshold priority	LSM (kPa)	Sensitivity	Specificity	PPV	NPV
Early Disease detection								
	VCTE[43]	0.79	High Sensitivity	5.6	0.9	0.44	0.62	0.81
			Balanced	8.6	0.66	0.80	0.78	0.70
			High Specificity	11.9	0.40	0.90	0.80	0.59
F 2	MRE[38]	0.92	Balanced	2.97	0.85	0.85	0.80	0.89
Cirrhosis detection								
	VCTE[43]	0.93	High Sensitivity	12.1	0.90	0.82	0.34	0.99
F = 4			Balanced	13.1	0.89	0.86	0.39	0.99
			High Specificity	14.9	0.69	0.90	0.41	0.59
	MRE[38]	0.94	Balanced	4.7	0.80	0.86	0.41	0.97

F, fibrosis stage; AUROC, area under the receiver operator characteristic; LSM, liver stiffness measurement; PPV, positive predictive value; NPV, negative predictive value; VCTE, vibration-controlled transient elastography; MRE, magnetic resonance elastography

Table 2:

Advantages and Limitation of Common Non-invasive Tests for the Diagnosis and Monitoring of Non-Alcoholic Fatty Liver Disease.

Non-invasive test	Distinguish NASH from simple steatosis	Clinical context	Advantages	Limitations
Alanine Aminotransferase (ALT)	No	-Often used to identify individuals at risk for NAFLD. -Monitor response to therapy	-Widely available -Inexpensive	-Low sensitivity and specificity for NASH and fibrosis
Cytokeratin 18	Maybe	Research only, not available clinically	-May be able to distinguish those with NASH compared to simple steatosis, though more research is needed.	-Variability in cut-offs used in various studies -Not clinically available
NAFLD Fibrosis score	No	-Used to estimate an individual's risk for advanced fibrosis -Possible role in monitoring response to therapy	-Easy to calculate -Clinical information for the score is often available	-Large number of individuals fall in the indeterminate range -Different cut-off values needed for younger or older participants -Limited usefulness in the general population
Fibrosis-4	No	-Used to estimate an individual's risk for advanced fibrosis -Possible role in monitoring response to therapy	-Easy to calculate -Clinical information for the score is often available	-Large number of individuals fall in the indeterminate range -Various cut-offs used in studies -Limited usefulness in the general population
Vibration-Controlled Transient Elastography (VCTE; Fibroscan)	Maybe (in combination AST)	-Performs best at ruling out advanced fibrosis -Used to quantify liver fat -Possible role in monitoring response to therapy	-Performed in liver clinic - Simultaneously quantify fat (CAP) - Integrated quality control -Larger area of liver assessed -No prior experience with ultrasound required -portable options available	-Failure if narrow rib spaces -Failure if large ascites -Only measures CAP and LSM -Less cost effective if also need ultrasound
Supersonic shear imaging (SSI)	No	-Performs best at ruling out advanced fibrosis -Possible role in monitoring response to therapy	-Not impacted by rib spaces -Ok with large ascites -Evaluate other features of portal hypertension -Cost effective if also need ultrasound	-Performed in radiology -Does not quantify fat -Quality control not integrated -Smaller area of liver assessed -Lack of portability -Requires ultrasound expertise
Acoustic radiation force impulse (ARFI)	No	Same as SSI	Same as SSI	Same as SSI
Magnetic Resonance Elastography (MRE)	No	-Able to distinguish between fibrosis stages -Rules in or out advanced fibrosis -Possible role in monitoring response to therapy	-Overall best performance -Ok with large ascites -Can be easily performed with other techniques to quantify liver fat -Largest area of the liver assessed	-Performed in radiology -Performed at a limited number of centers -Quality control not integrated -Lack of portability Cost

NAFLD, Nonalcoholic Fatty Liver Disease; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; AST, aspartate aminotransferase