Received: 1 December 2022 | Revised: 7 February 2023 | Accepted: 20 February 2023

DOI: 10.1111/liv.15555

Liver INTERNATIONAL WILEY

REVIEW

The use of current knowledge and non-invasive testing modalities for predicting at-risk non-alcoholic steatohepatitis and assessing fibrosis

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Handling Editor: Alejandro Forner

Abstract

There is ongoing recognition of the burden of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), with fibrosis being the most important histological feature that is associated with progression to cirrhosis and the occurrence of major adverse liver outcomes. Liver biopsy is the gold standard applied to detect NASH and determine the stage of fibrosis, but its use is limited. There is a need for non-invasive testing (NIT) techniques to identify patients considered at-risk NASH (NASH with NAFLD activity score > 4 and ≥ F2 fibrosis). For NAFLD-associated fibrosis, several wet (serological) and dry (imaging) NITs are available and demonstrate a high negative predictive value (NPV) for excluding those with advanced hepatic fibrosis. However, identifying at-risk NASH is more challenging; there is little guidance on how to use available NITs for these purposes, and these NITs are not specifically designed to identify at-risk NASH patients. This review discusses the need for NITs in NAFLD and NASH and provides data to support the use of NITs, focusing on newer methods to non-invasively identify at-risk NASH patients. This review concludes with an algorithm that serves as an example of how NITs can be integrated into care pathways of patients with suspected NAFLD and potential NASH. This algorithm can be used for staging, risk stratification and the effective transition of patients who may benefit from specialty care.

KEYWORDS
At-risk, non-alcoholic steatohepatitis, non-invasive testing

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of steatosis in ≥5% of hepatocytes, without significant alcohol consumption or other known causes of steatosis.[1] NAFLD is subdivided into two primary subtypes, the fairly benign non-alcoholic fatty liver (NAFL) and the more severe, progressive form termed

Abbreviations: AACE, American Association of Clinical Endocrinology; AASLD, American Association for the Study of Liver Diseases; AGA, American Gastroenterological Association; aHR, adjusted hazard ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; CAP, controlled attenuation parameter; CLD, chronic liver disease; cT1, corrected T1; EASL, European Association for the Study of the Liver; FAST, FibroScan-aspartate aminotransferase; FIB4, Fibrosis-4 index; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; MALOs, major adverse liver outcomes; MAST, MRI-aspartate aminotransferase; MEFIB, combination of MRE and FIB4; MELD-Na, Model for end-stage liver disease-sodium; MetS, metabolic syndrome; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; NAFL, non-alcoholic fatty liver; NAFLD, Non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, Non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; NIT, non-invasive testing; NPV, negative predictive value; T2DM, type-2 diabetes mellitus; VCTE, vibration-controlled transient elastography.

non-alcoholic steatohepatitis (NASH), defined histologically by steatosis in ≥5% of hepatocytes, lobular inflammation and liver cell injury (hepatocyte 'ballooning'), with or without fibrosis.[1] Until recently, there was a lack of large prospective studies to establish the prevalence of NAFLD and NASH. A recent study used magnetic resonance imaging-based proton density fat fraction (MRI-PDFF) to screen for NAFLD and the gold standard reference biopsy[1] to screen for NASH. The cohort included 664 asymptomatic, middle-aged adults (mean age: 56 ± 6.4 years, 50% male, mean body mass index [BMI]: 30.48 ± 5.46 kg/m^2, 52% obese). Investigators determined that the overall prevalence of NAFLD was 38% and that of NASH by biopsy was 14%.[2] These estimates are higher than those previously reported; in 2016, the global prevalence rates of NAFLD and NASH were estimated at 25% and 1.5–6.45% respectively.[3] A more recent meta-analysis suggests that the global prevalence of NAFLD is approximately 30%.[4] The growing prevalence of NAFLD and NASH may partly be owing to the increasing prevalence of associated comorbidities. Metabolic comorbidities associated with NAFLD and NASH include obesity, type-2 diabetes mellitus (T2DM), dyslipidaemia, hypertension and metabolic syndrome (MetS).[3,5] The prevalence of NAFLD in patients with T2DM or MetS is estimated at over 50%,[6] but when confirmed using biopsy, it has been found to be over 90% in these populations.[7]

There are significant knowledge gaps regarding the diagnosis, natural history and treatment of NAFLD, but it is known that NAFLD is considered 'dichotomous'. NAFL poses lower risk to the patient, from a liver-related perspective, while NASH may lead to progressive hepatic fibrosis, liver cirrhosis, liver failure and hepatocellular carcinoma (HCC).[1,8,9] An analysis of Medicare claims between 1999 and 2012 found that NAFLD was the most common cause of chronic liver disease (CLD) in all ethnic groups combined (52%).[9] The most important histological feature of NAFLD associated with long-term mortality is fibrosis,[1] and NAFLD has been found to be one of the most common causes of cirrhosis leading to liver transplants, second only to alcohol-associated liver disease.[10] Another study found that the number of new waitlisted liver transplant registrants with NAFLD increased by 170% from 2004 to 2013.[11] NASH has become the most common cause of cirrhosis in adults,[9] the second leading cause for waitlisting and liver transplants in all patients and the first leading cause for waitlisting and liver transplants among Asian, Hispanic and non-Hispanic white females.[11]

Identifying the presence of significant fibrosis (≥F2 fibrosis, NAFLD activity score [NAS] > 4) as a result of NASH in high-risk patients is of utmost importance, given its prognostic value and correlation with progression to cirrhosis and major adverse liver outcomes (MALOs). Liver biopsy is the gold standard applied to detect fibrosis, but its use is limited. Several non-invasive tests (NITs) are available for this purpose, which include 'wet' (i.e., NITs that use serum biomarkers to identify fibrosis) and 'dry' (i.e., NITs that use imaging to identify fibrosis). These NITS demonstrate high accuracy when used in combination, but staging and risk stratification of NASH are more challenging; there is no guidance on how to use available NITs for these purposes. This review discusses the need for NITs in NAFLD and NASH and provides data to support the use of NITs, focusing on newer methods of non-invasively identifying at-risk NASH patients (≥F2 fibrosis, NAS ≥4). This review concludes with an algorithm that serves as an example of how NITs can be integrated into care pathways of patients with NAFLD for better staging and risk stratification, as well as inform the referral decision for further evaluation.

Key Points

- Significant fibrosis (≥F2, NAS > 4) in NASH has prognostic value and is correlated with progression to cirrhosis and major adverse liver outcomes.
- The use of liver biopsies is limited and use of NITs for risk stratification is becoming increasingly more common.
- Data on NIS4, FAST score, MAST score and multimodality imaging with cT1 demonstrate efficacy in identifying this subgroup of patients.
- We propose an algorithm that uses validated NITs in combination and provides accessible, standardized, evidence-based, timely and testable recommendations.
- Algorithms such as this one could potentially eliminate the need for unnecessary biopsies in suspected NASH cases.

2 | NEED FOR NON-INVASIVE TESTING (NIT) IN NON-ALCOHOLIC FATTY LIVER DISEASE/NON-ALCOHOLIC STEATOHEPATITIS (NAFLD/NASH)

Patients with NASH and advanced hepatic fibrosis are at significant risk of complications, such as end-stage liver disease, HCC and liver transplant.[3,12–17] Data from cited studies suggest that the degree of fibrosis is a major driver of clinical outcomes. In fact, the adverse outcomes observed in patients with NASH could be chiefly driven by the concomitant presence of advanced fibrosis (or higher) and not NASH per se. A retrospective cohort study of 646 biopsy-proven NAFLD patients found that during a mean follow-up of 20 years, compared with controls, the risk of severe liver disease increased per stage of fibrosis (hazard ratio ranging from 1.9 in F0 to 104.9 in F4). Similar results were seen for overall mortality. Accounting for the presence of NASH did not increase the risk of liver-specific morbidity or overall mortality.[18] In a prospective study, 1773 adults with NAFLD were followed for a median of 4 years. All-cause mortality increased with increasing fibrosis stages (0.32 deaths per 100 person-years for stage F0 to F2 [no, mild, or moderate fibrosis], 0.89 deaths per 100 persons-years for stage F3 [bridging fibrosis], and 1.76 deaths per 100 person-years for stage F4 [cirrhosis]). The incidence of liver-related complications per 100 person-years increased with fibrosis stage (F0 to F2 vs. F3 vs. F4) as follows: variceal haemorrhage (0.00 vs. 0.06 vs. 0.70), ascites (0.04 vs. 0.52 vs. 1.20), encephalopathy

(0.02 vs. 0.75 vs. 2.39) and hepatocellular cancer (0.04 vs. 0.34 vs. 0.14).[19] A systematic review and meta-analysis, which included 1495 NAFLD patients with 17 452 patient-years of follow-up, found that as fibrosis advances in stage (on a scale of 0 to 4), the risk of liver-related mortality exponentially increases. The quantitative risks of liver-related mortality were 1.22 for stage-1 fibrosis, 4.85 for stage 2, 8.86 for stage 3 and 21.6 per 1000 patient-years of follow-up for stage 4. The risk of liver-related death was found to be statistically higher only after progression to stage-2 fibrosis or higher.[14] Other studies[15,20] also demonstrated the significance of progression after F2; Younossi et al[20] and Ekstedt et al[15] demonstrated that NAFLD patients with advanced fibrosis (stages 3–4) were at an increased risk of all-cause mortality, but this increased risk was not observed among those with early-stage fibrosis (stages 1–2).

The above data indicate the importance of identifying NAFLD patients who are at risk of such progression. Additionally, NASH progresses to advanced fibrosis and cirrhosis more frequently than NAFL,[16] with one study demonstrating that nearly a quarter of histologically confirmed NASH patients progressed from F3 fibrosis to cirrhosis at a median of 29 months.[21] Based on these findings, NASH patients with ≥F2 fibrosis are considered to have the most urgent need for identification and interventions, and they are labelled as 'at-risk NASH'. The NAS, which is a composite sum of the histological scores for steatosis, hepatocellular injury as evidenced by ballooning, and lobular inflammation, is also used to evaluate NASH activity.[22] Liver biopsy data indicate that a higher NAS at the baseline is correlated with a high probability of fibrosis stage progression after 1 year or more,[23] and higher rates of spontaneous disease regression have been observed in both treated and untreated patients with milder NASH severity (NAS 3) compared with patients with higher activity (NAS ≥4) at the baseline.[24] Patients with a NAS score ≥4 and fibrosis ≥2 are also considered to have at-risk NASH and have become the target population for pharmacological treatment and enrolment in phase-3 clinical trials for NASH treatments.[12,23,25–27]

Liver biopsy remains the gold standard method for fibrosis staging in NAFLD, diagnosis of NASH and identification of at-risk NASH.[1,12] As detailed in Figure 1,[12,28,29] liver biopsy is associated with many limitations. Therefore, in recent years, significant attention has been paid to the development and use of NITs.

3 | NITS TO DETERMINE FIBROSIS STAGE AND PREDICT MAJOR ADVERSE LIVER OUTCOMES (MALOS) IN NAFLD

3.1 | Staging fibrosis in NAFLD

One of the necessary steps in assessing patients with NAFLD and NASH is to determine their fibrosis stage.[30] Given that liver biopsies are impractical given the large number of NAFLD patients and are associated with many other limitations (Figure 1), several NITs are becoming widely used for this purpose. A brief review of wet NITs is provided in Table 1,[31–37] and a detailed overview of dry NITs is presented in Table 2.[38]

With regard to wet NITs, the Fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS) (Table 1) are the most extensively utilized. FIB-4 and NFS have been highlighted in the AASLD guidance document as 'clinically useful tools for identifying patients with NAFLD with

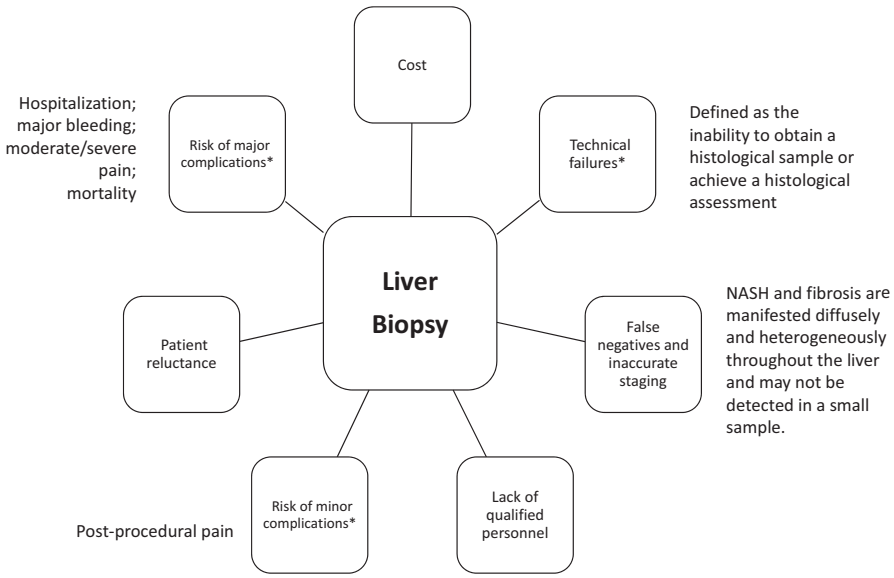

FIGURE 1 Limitations associated with liver biopsies. *In a systematic review and meta-analysis of 30 studies that report complications resulting from 64 356 percutaneous liver biopsy procedures in patients with chronic liver disease, the incidence of major complications was 2.44% (95% CI: 0.85–6.75), including hospitalization in 0.65% (95% CI: 0.38–1.11), major bleeding in 0.48% (95% CI: 0.22–1.06), moderate/severe pain in 0.34% (95% CI: 0.08–1.37) and with mortality in 0.01% (95% CI: 0.00–0.11). Approximately, 1 in 10 patients experienced post-procedural pain or other minor complications. Technical failure, which is the inability to obtain a histological sample or achieve a histological assessment, may occur in approximately 1% of liver biopsy procedures.[25]

higher likelihood of having advanced hepatic fibrosis'.[1] Data indicate that these tests accurately exclude advanced fibrosis with high specificity (FIB score < 1.3, NFS < -1.455)[39–41] and thus should be used for first-line triaging, and if appropriate, linkage to specialty care.[12] The enhanced liver fibrosis (ELF) score is a wet NIT that was granted marketing authorization in 2021.[42] ELF is an extracellular matrix marker set consisting of tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type-III procollagen and hyaluronic acid. Unlike FIB-4 and NFS, this serum biomarker requires a specialized laboratory service that provides the score after analysing a patient's blood sample.[43]

The use of these wet NITs is not without caveats. While the formulas for diagnostic scores include liver transaminases, it is worth noting that abnormal transaminase levels do not affect the diagnostic capabilities of these tests. One study investigated the diagnostic performances of FIB-4 and NFS in the estimation of advanced fibrosis comparing patients with normal and elevated transaminases and found that both NITs showed acceptable diagnostic performance in the exclusion of advanced fibrosis in both populations.[44] Furthermore, specific subgroups may need further attention. Accuracy of these wet NITs is affected by age and age-specific cut-offs have been proposed to retrieve more accurate results. One study demonstrated that the specificity for advanced fibrosis using the FIB-4 and NFS declined with age, becoming unacceptably low in those aged ≥65 years. Investigators therefore designed and validated new cut-offs in this patient population to exclude advanced fibrosis (FIB score < 2, NFS < 0.12).[45] The fact that patients with diabetes mellitus pose a higher risk for adverse outcomes in NAFLD coupled with the limited utility demonstrated by FIB-4 and NFS demonstrated in these patients indicates that this patient population needs separate attention.[46–48] Finally, the clinical utility of these NITs in morbidly obese patients remains controversial because of some inconsistencies in the published literature. For example, one study found that FIB-4 and NFS can confidently be used to exclude advanced fibrosis in overweight, obese and severely obese patients,

TABLE 1 'Wet' NITs that use serum biomarkers and algorithms to identify fibrosis.

Test	Factors used to identify fibrosis
FIB4[37]	Age, platelets, AST and ALT
NFS[31]	Age, platelets, AST/ALT ratio, albumin, BMI, and whether the patient has impaired fasting glucose/diabetes
APRI[32]	AST and upper limits of normal and platelet counts
Hepascore®[35]	Bilirubin, gamma-glutamyltransferase, hyaluronic acid, alpha (2)-macroglobulin, age and sex
FibroTest® (FibroSure® in the US)[36]	a2-macroglobulin, apolipoprotein A1, total bilirubin, haptoglobin and g-glutamyl transferase
FibroMeter®[34]	Age, weight, platelet count, AST, ALT, ferritin and glucose
ELF[33]	Tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type-III procollagen and hyaluronic acid A specialized laboratory is needed to assess these analytes.

Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; ELF, enhanced liver fibrosis; FIB4, Fibrosis-4 index; NFS, NAFLD fibrosis score.

TABLE 2 'Dry' NITs that use imaging to identify fibrosis.

Test	Factors used to identify fibrosis	Advantages	Disadvantages
VCTE	The velocity of the wave that travels through the liver indicates LSM; the higher the LSM, the greater the degree of liver stiffness.	Widely used, broadly validated, well-defined quality criteria; good reproducibility; good performance in cirrhosis (AUROC > 0.9); prognostic value for compensated cirrhosis	Affected by obesity, ascites, operator experience; can only assess superficial liver regions; risk of false positives
SWE	The propagation velocity of the shear wave that travels through the liver is correlated with the elasticity of tissue; a higher velocity indicates increasing LSM.	Larger region of interest than VCTE, chosen by operator; measures liver stiffness in real time; Prognostic value for compensated cirrhosis; Detects significant fibrosis and cirrhosis	Affected by operator experience; Risk of false positives
MRE	Propagates acoustic shear waves into the liver and computes cross-sectional images using a mathematical algorithm, thereby allowing detection of fibrosis	Performed on standard MR scanner; whole liver study; superior to TE in pts with ascites and obesity; performs well for early stages of fibrosis; predicts clinical outcomes[38]; can diagnose cirrhosis	Not useful in iron overload; expensive
ARFI	An ultrasound-based technique that evaluates the wave propagation speed and allows the assessment of tissue stiffness	Allows use of conventional ultrasound; shear wave can be localized to avoid blood vessels and ribs	Cut-off values for advanced fibrosis vary; obese patients demonstrate increased failures

Abbreviations: ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; CAP, controlled attenuation parameter; CT, computed tomography; FAST, FibroScan-AST; LSM, liver stiffness measurement; MRE, magnetic resonance elastography; SWE, shear wave elastography; TE, transient elastography.

but are not clinically useful in lean and morbidly obese patients.[49] Conversely, another study found that FIB-4 score is an accurate predictor of advanced fibrosis in NAFLD throughout all BMI stages, whereas NFS tends to overestimate fibrosis in morbidly obese NAFLD patients.[50]

As previously noted, these simple NITs are useful for ruling out advanced fibrosis, but are not adequate for detecting advanced fibrosis. One study suggests that combining two wet biomarkers (ELF and FIB-4) show utility. The ELF test performed well in identifying patients with NAFLD who are at increased risk of advanced fibrosis and the FIB-4 reliably assessed the presence or absence of advanced fibrosis among patients with NAFLD.[51] Stepwise approaches may also involve wet and dry NITs (Table 2). Data indicate that the sequential use of the age-adjusted FIB-4 and liver stiffness measures (LSMs) yield the least uncertainty (5.3%) in classifying disease severity with the highest diagnostic accuracy (81%) among a variety of NIT combinations.[52] One study found that a paired combination of the FIB-4 and LSM (<8.8 kPa for exclusion of advanced fibrosis and >10.9 kPa for inclusion of advanced fibrosis) was able to diagnose patients with advanced fibrosis with the highest diagnostic accuracy.[53]

The American Association for the Study of Liver Diseases (AASLD),[1] the American Gastroenterological Association (AGA),[54] the American Association of Clinical Endocrinology (AACE),[55] and the European Association for the Study of the Liver (EASL)[56] all recommend that patients with NAFLD undergo fibrosis risk stratification using the wet and the dry NITs in combination or sequentially. Liver biopsy is the reference standard and should be considered in case of uncertainty regarding the contributing causes of liver injury and/or the stage of liver fibrosis.[57]

3.2 | Predicting MALOs in NAFLD

In addition to serving as accurate biomarkers of liver fibrosis, NITs have also demonstrated utility in predicting the likelihood of developing MALOs in NAFLD. One study evaluated the prognostic accuracy of the Fibrosis-4 index (FIB4) and vibration-controlled transient elastography (VCTE), compared with liver biopsy, for the prediction of MALOs in NAFLD. In total, 1057 patients with NAFLD and baseline FIB4 and VCTE, 594 of whom also had a baseline liver biopsy, were included in a multicentre cohort. The results showed that FIB4 and VCTE stratified the risk of MALOs in NAFLD. Compared with patients with FIB4 < 1.30, those with FIB4 ≥ 1.30 and VCTE < 8.0 kPa had a similar risk of MALOs (adjusted hazard ratio [aHR]: 1.3; 95% CI: 0.3–6.8), whereas the risk of MALOs significantly increased in patients with FIB4 ≥ 1.30 and VCTE 8.0–12.0 kPa (aHR: 3.8; 95% CI: 1.3–10.9), and even more for those with FIB4 ≥ 1.30 and VCTE > 12.0 kPa (aHR: 12.4; 95% CI: 5.1–30.2). Additionally, VCTE demonstrated prognostic accuracy similar to that of liver biopsy; in the biopsy subgroup, Harrell's C-indexes of histological fibrosis staging and VCTE were not significantly different (0.932 [0.910–0.955] versus 0.881 [0.832–0.931], respectively, $p = .164$).[58]

MRI-based techniques have also been evaluated for predicting MALOs. A pooled meta-analysis of 1707 NAFLD patients demonstrated that liver stiffness assessed by magnetic resonance elastography (MRE) was associated with liver-related events, as the 3-year risk of incident HCC was 0.35% for MRE < 5 kPa, 5.25% for MRE 5–8 kPa, and 5.66% for MRE ≥8 kPa respectively. The combination of MRE and FIB4 (MEFIB) also had an excellent NPV for hepatic decompensation. Specifically, the MEFIB index (defined as positive when MRE ≥3.3 kPa and FIB4 ≥ 1.6) had a robust association with the primary outcome, with a hazard ratio of 20.6 (95% CI: 10.4–40.8, $p < .001$) and a negative MEFIB had a high NPV for the primary outcome, 99.1% at 5 years.[59] A multicentre retrospective study that included 320 NAFLD patients found that liver stiffness measured by MRE with a cut-off value of ≥6.48 kPa was associated with decompensation and mortality, and specific MRE cut-off values were predictive of individual clinical liver events. The odds of decompensation increased as liver stiffness increased (odds ratio [OR]: 3.28, $p < .001$), and increased liver stiffness was associated with ascites, hepatic encephalopathy, oesophageal variceal bleeding and mortality (median: 7.10, 8.85, 10.15 and 10.15 kPa respectively).[60] A separate study analysed 829 adults with NAFLD who underwent MRE between 2007 and 2019. Of the 639 patients without cirrhosis, 20 developed cirrhosis after a median follow-up of 4 years. The baseline liver stiffness measurement (LSM) by MRE was predictive of future cirrhosis development (age-adjusted HR = 2.93; 95% CI: 1.86–4.62, $p < .0001$) per 1 kPa increment (C-statistic = 0.86) and useful as a guide for the timing of longitudinal non-invasive monitoring (5, 3 and 1 years for LSM of 2, 3 and 4–5 kPa respectively). Of the 194 patients with compensated cirrhosis, 81 developed decompensation or died after a median follow-up of 5 years. The baseline LSM was predictive of future decompensation or death (HR = 1.32; 95% CI: 1.13–1.56, $p = .0007$) per 1 kPa increment after adjusting for age, sex and Model for End-Stage Liver Disease-Sodium (MELD-Na). The 1-year probability of future decompensation or death from cirrhosis with baseline LSM of 5 kPa versus 8 kPa is 9% versus 20% respectively.[61]

Iron-corrected T1 mapping (cT1) from MRI has also been linked to incidence of MALO. In a study of 197 chronic liver disease patients conducted at the University of Oxford, 14 new clinical events (including ascites, variceal bleeding, hepatic encephalopathy, HCC, liver transplantation and mortality) and 11 deaths occurred over a course of 693 patient-years. A liver cT1 threshold of 825 ms predicted clinical outcomes with a hazard ration (HR) of 9.9 (1.29–76.4). This was equivalent to the HR of the Ishak fibrosis score from biopsy and superior to the predictive score for liver stiffness from VCTE after accounting for the technical failure and unreliability of the latter.[62]

3.3 | NITs to diagnose At-Risk NASH

Given the aforementioned consequences of the progression of NASH,[9,11] it is becoming increasingly clear that NASH patients who are at the highest risk of progressive liver disease (i.e., fibrosis ≥F2

and/or NAS ≥4) must be identified early in the disease course.[12] In the USA, approximately, 4.5 million people might have advanced fibrosis related to NASH[63] but unfortunately, go unrecognized. Most patients with end-stage liver disease secondary to NASH have no previous diagnosis of liver disease. This is related to the lack of screening guidance for high-risk individuals[64] and the available NITs (Tables 1 and 2) not being specifically designed to identify at-risk NASH.[65] Recent efforts to non-invasively identify this subgroup of patients and to reduce the need for unnecessary biopsies are explored in this section. These include the NIS4, FibroScan-aspartate aminotransferase (FAST) score, MRI-aspartate aminotransferase (MAST) score and iron-corrected T1 (cT1) from quantitative MR imaging.

3.4 | NIS4

A blood-based diagnostic test to non-invasively rule in and rule out at-risk NASH was recently developed and validated. The derived panel, called NIS4, comprises four independent NASH-associated biomarkers: miR-34a-5p (circulating concentrations are associated with NASH histology), alpha-2 macroglobulin (shown to promote liver fibrosis through the inhibition of matrix protein catabolism in inflammatory or injured liver), YKL-40 (a biomarker of hepatic fibrosis produced by activated macrophages) and glycated haemoglobin (a classic marker of glycaemic control, which, when altered, has been shown to drive fibrosis in NASH). The diagnostic performance of the panel was assessed using the area under the receiver operating characteristic curve [AUROC] analysis (0.80, 95% CI: 0.73–0.85); the diagnostic performance in the external validation cohorts was not influenced by age, sex, BMI, or aminotransferase concentrations. In the pooled validation cohort, the patients whose NIS4 value was less than 0.36 were classified as not having at-risk NASH (ruled out) with 81.5% (95% CI: 76.9–85.3) sensitivity, 63.0% (95% CI: 57.8–68.0) specificity and an NPV of 77.9% (95% CI: 72.5–82.4), whereas those whose NIS4 value was more than 0.63 were classified as having at-risk NASH (ruled in) with 87.1% (95% CI: 83.1–90.3) specificity, 50.7% (95% CI: 45.3–56.1) sensitivity, and a positive predictive value (PPV) of 79.2% (95% CI: 73.1–84.2). The authors concluded that NIS4 provided an effective way to non-invasively rule in or rule out at-risk NASH in patients with metabolic risk factors and suspected disease.[65]

3.5 | FibroScan-Aspartate Aminotransferase (FAST) score

Newsome and colleagues sought to develop an algorithm to identify at-risk NASH patients to qualify these patients for recruitment to clinical trials. The model, designated as the FAST score, combined the LSM by VCTE as a marker of fibrosis, the controlled attenuation parameter (CAP) as a marker of steatosis, and aspartate aminotransferase (AST) as a marker of disease activity. The derivation cohort consisted of 350 patients with suspected NAFLD attending liver clinics in England, whose performance was deemed satisfactory (C-statistic: 0.80; 95% CI: 0.76–0.85) and was well calibrated. In external validation cohorts, the calibration of the score was satisfactory, and the discrimination was good across the full range of validation cohorts (C-statistic range: 0.74–0.95, 0.85; 95% CI: 0.83–0.87 in the pooled external validation cohort; $n = 1026$). The cut-off values were 0.35 for a sensitivity of 0.90 or greater and 0.67 for a specificity of 0.90 or greater in the derivation cohort, leading to a PPV of 0.83 (84/101) and an NPV of 0.85 (93/110). In the external validation cohorts, the PPV ranged from 0.33 to 0.81, and the NPV ranged from 0.73 to 1.0. The FAST score was determined to be an efficient way to non-invasively identify patients at risk of progressive NASH for clinical trials or treatments when they become available, thereby reducing unnecessary liver biopsies in patients who were unlikely to have a significant disease.[66]

3.6 | MRI-Aspartate aminotransferase (MAST) score

The NITs detailed above show promise in identifying at-risk NASH but do not include MRI-based techniques, which are the most commonly used primary and secondary endpoints in early-phase NASH clinical trials. Noureddin and colleagues developed the MAST score, which incorporates MRI-PDFF and MRE, techniques that have proven better than VCTE in identifying steatosis and staging fibrosis, respectively, to identify at-risk NASH. The third variable, AST, was selected for its highest balanced accuracy out of nine other variables (alanine aminotransferase [ALT], AST, AST/ALT ratio, albumin, platelets, diabetes status, sex, age and body mass index) using logistic regression. In the validation cohort ($n = 244$), the MAST score demonstrated high performance and discrimination (AUC: 0.93; 95% CI: 0.88–0.97). In the validation cohorts, the 90% specificity cut-off value of 0.242 corresponded to a sensitivity of 75.0%, a PPV of 50.0% and an NPV of 96.5%, whereas the 90% sensitivity cut-off value of 0.165 corresponded to a specificity of 72.2%, a PPV of 29.4% and an NPV of 98.1%. The MAST score also outperformed other NITs in identifying at-risk NASH; compared with the NAFLD fibrosis score (NFS) and FIB4, the MAST score resulted in fewer patients having indeterminate scores and an overall higher AUC, and compared with FAST, MAST exhibited a higher AUC and overall better discrimination.[67]

3.7 | Multiparametric MRI using corrected T1 (cT1)

Iron-corrected T1 mapping (cT1) of liver tissue is a diagnostic enrichment biomarker that can be used for the recruitment of patients to clinical trials, in conjunction with clinical risk factors, to identify participants who are more likely to have at-risk NASH.[68] T1 MR relaxation times of the liver lengthen in proportion to extracellular

fluid, which provides a measure of tissue inflammation and fibrosis. However, increasing amounts of intrahepatic iron decrease the T1 MR relaxation times and confound the assessment. In this regard, cT1 is an algorithm that removes the bias introduced by elevated iron on T1.[69] Multiparametric MRI uses T2 relaxation times to quantify and correct liver iron levels via an algorithm. The resulting iron-cT1 score is correlated with all features of the NAS (steatosis, inflammation and ballooning) and with the Kleiner-Brunt fibrosis stage, as determined by liver biopsy.[70]

The results produced by multiparametric MRI are correlated with histopathological features of NASH[2,70] and detect changes in inflammation and fibrosis,[70] even in the absence of ALT/AST changes.[71] In a study of 145 NAFLD patients from Japan, cT1 was directly compared with measures of liver stiffness from VCTE and MRE for the identification of at-risk NASH and was superior to each with AUC for cT1 of 0.74 and AUCs for VCTE and MRE of 0.64 and 0.66 respectively.[72] Previous data have demonstrated that MRI-PDFF is strongly correlated with histologic steatosis but has limited utility in identifying at-risk NASH because as fibrosis progresses, liver fat decreases.[70,73] The clinical utility of cT1 versus MRI-PDFF or MRE for identification of at-risk NASH has been investigated and reported. Additionally, cT1 was compared with PDFF using pooled data from five clinical studies ($n = 543$) with suspected NAFLD patients. cT1 was found to be a better non-invasive technology than PDFF to identify NASH patients at greatest risk of disease progression. The diagnostic accuracy of cT1 in identifying patients with high-risk NASH was good (AUROC: 0.78; 95% CI: 0.74–0.82), superior to MRI-PDFF (AUROC: 0.69; 95% CI: 0.64–0.74) and not substantially improved by combining it with MRI-PDFF (AUROC: 0.79; 95% CI: 0.75–0.83). By using a cut-off value of 875 ms or higher to rule in the presence of at-risk NASH, cT1 had a sensitivity of 59%, specificity of 81%, PPV of 55% and NPV of 83%. On the other hand, by using a cut-off value of 800 ms or lower to rule out the presence of at-risk NASH, cT1 had a sensitivity of 86%, specificity of 56%, PPV of 45% and NPV of 91%.[74]

4 | CONCLUSION

4.1 | A proposed algorithm using various NITs to identify at-risk NASH

Clinical care pathways with careful explication of each step in the screening, diagnosis and treatment have been shown to improve the quality of healthcare delivery in other areas of medicine. Kanwal and colleagues recently developed a clinical care pathway to assist clinicians in diagnosing and managing NAFLD with clinically significant fibrosis (stages F2–F4), based on the best available evidence. This pathway begins with identifying whether a patient is at the greatest risk of NAFLD/NASH-related fibrosis (i.e., if the patient has T2DM or two or more metabolic risk factors or there is incidental finding of hepatic steatosis or elevated aminotransferases). If the patient falls into one of these three categories, further testing is indicated to determine if the patient is at an indeterminant risk (FIB4: 1.3–2.67), which warrants an LSM. Based on the LSM, the patient is classified as being at low (<8 kPa), indeterminant (8–12 kPa), or high (>12 kPa) risk and linkage to care and management strategies is recommended accordingly.[54]

Algorithms from the three major societies suggest liver biopsy for patients at intermediate/indeterminate risk of advanced liver fibrosis,[54–56] but now, it may be possible to incorporate NITs into an algorithm for use in these patients to potentially minimize the need for biopsy. Such an algorithm has been developed. It uses validated NITs in combination (Figure 2) and has goals similar to those of the aforementioned clinical care pathway, providing accessible, standardized, evidence-based, timely and testable recommendations that will allow clinicians to care for a rapidly growing population of patients.[54]

Per the algorithm, patients with VCTE-derived LSM <8 kPa are unlikely to have at-risk NASH, and the focus should be on lifestyle modifications to induce weight loss. Those with LSM >14 kPa in combination with elevated FIB4 > 3.25 have very high PPV of having advanced disease and should be followed up by a specialist. Those with LSM between 8 and 12 kPa should undergo multiparametric MRI to obtain a cT1 score to detect the presence of inflammation and fibrosis, as well as at-risk NASH. A cT1 score < 800 ms indicates isolated steatosis, while a cT1 score ≥ 800 ms is consistent with NASH. A patient whose cT1 score is ≥875 ms likely has at-risk NASH. Per the recently updated AGA clinical guidance for the management of lean NAFLD, the cT1 score may be used instead of or prior to liver biopsy in all patients at intermediate/high risk of fibrosis, but this decision should be made by a hepatologist.[57]

A UK decision analytic model found that the inclusion of multiparametric MRI, either as an adjunct to or replacement of transient elastography, in the diagnostic pathway of NAFLD may lead to cost savings by reducing the number of liver biopsies.[75] Another study out of Europe concluded that for the risk stratification of NAFLD, multiparametric MRI was cost-effective, and combined with transient elastography, had the lowest cost per correct diagnosis.[76] It is important to emphasize that these cost effectiveness analyses are specific to their healthcare systems and may not apply in other parts of the world.

This algorithm is an example of how NITs can be integrated into the clinical care pathway of patients at risk of NAFLD for better staging and risk stratification, as well as inform the referral decision for further evaluation. Alternative algorithms that may incorporate other tests such as MAST can be used based on clinical availability. These algorithms could potentially eliminate the need for unnecessary biopsies in suspected NASH cases.

ACKNOWLEDGEMENTS

Rachel E. Bejarano, PharmD, provided medical writing assistance.

FUNDING INFORMATION

This manuscript was supported by Perspectum. The selection of the authors and the creation of this manuscript were done independently, in which Perspectum did not play a role.

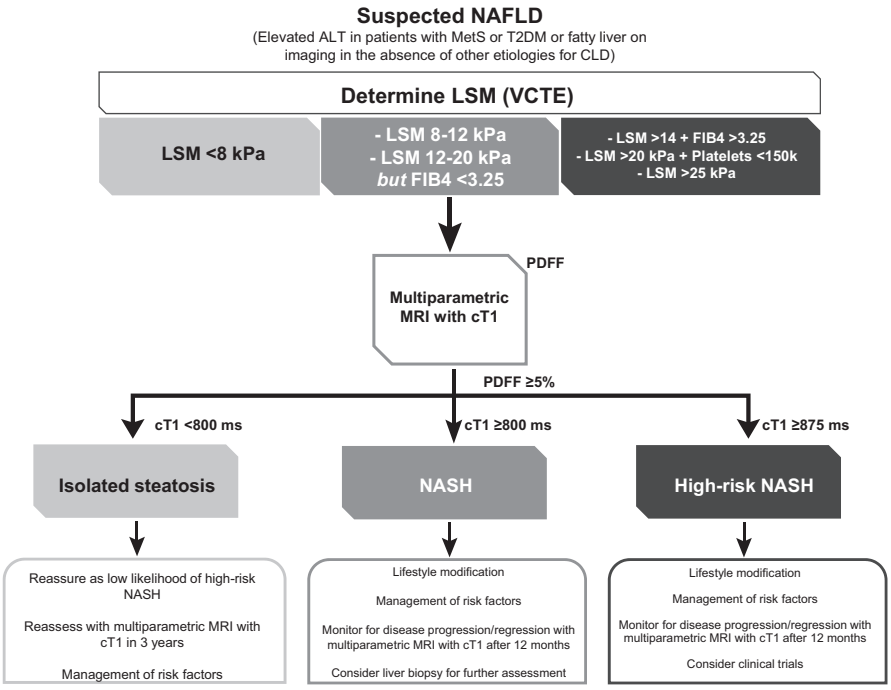

FIGURE 2 A Proposed Algorithm for Specialists to Identify At-Risk NASH Using a Combination of NITs, Including LSM, FIB4 and cT1. This algorithm is intended for specialists who, at this point in time, are the best resources to interpret cT1. ALT, alanine aminotransferase; CLD, chronic liver disease; cT1, corrected T1; FIB4, Fibrosis-4 index; HCC, hepatocellular carcinoma; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; LSM, liver stiffness measurement; PDFF, proton density fat fraction; T2DM, type-2 diabetes mellitus; VCTE, vibration-controlled transient elastography.

CONFLICT OF INTEREST STATEMENT

Marcelo Kugelmas; Advisory Board: Gilead, Abbvie, Echosens, Intercept, Madrigal; Speakers Bureau: Gilead, Abbvie, Echosens, Intercept; Consultant: Gilead, Abbvie, Echosens, Madrigal, Topography Health, Intercept; Grants/Research Support: Madrigal, Genentech, Intercept, Gilead, Ionis, Bio89, Akero, Genfit, Viking, NorthSea, Celgene, Enanta, HighTide, Novartis, Tobira. Mazen Noureddin; Advisory Board: Altimmune, BI, BMS, 89BIO, EchoSens, Gilead, GSK, Merck, Novo Nordisk, OWL, Pfizer, Roche diagnostic and Siemens, Terns and Takeda. Principal Investigator for a Drug Study: Allergan, Akero, BMS, Gilead, Galectin, Genfit, Conatus,Corcept, Enanta, Madrigal, Novartis, Novo Nordisk, Shire, Terns, Viking and Zydus. Stockholder: Anaetos, Rivus Pharma, CIMA, ChronWell and Viking. Editorial Board: Gastroenterology. Associate Editor: Clinical Gastroenterology and Hepatology. Federal funding: NIH/NIDDK. Nadege Gunn; Research funding: Axcella, Gilead, Novo Nordisk, Eli Lilly, 89bio, Sagimet, Merck, Novo Nordisk, Helio Health, Madrigal, Akero, Glympse Bio, Galectin. Manal Abdelmalek; Advisory role: Madrigal, Intercept, Bristol-Myers Squibb, NGM Bio, Merck, Inventiva, 89bio, SonicIncytes, Hanmi, Novo Nordisk. Consultant: Hanmi, Inventiva, 89bio. Research funding: Intercept, Allergan, Madrigal, Genfit, Viking, Hanmi, Bristol-Myers Squibb, NGM Bio, Celgene, Boehinger-Ingelheim, Genentech, Target NASH, Novo Nordisk, Inventiva, Enanta, Enyo, Poxel, Durect, Galmed. Honoraria: Clinical Care Options, Fishawack, Inc., Medscape, Terra Firma, Inc., CLDF. Editorial Board: Hepatology. Kimberly Brown; Advisory role: Madrigal, Intercept, Salix, Gilead, Mallinckrodt. Consultant: Abbvie. Research funding: Salix, Eurofins. Honoraria: CLDF, Gilead, Mallinckrodt, Salix, Intercept, Madrigal. Editorial Board: Liver Transplantation. Board Membership: AASLD Foundation Board. Zobair Younossi, MD; Research funding and/or consultant: Gilead Sciences, Intercept, BMS, NovoNordisk, AstraZeneca, Siemens, Quest, Madrigal, Merck, Abbott. Naim Alkhouri. Advisory role: AbbVie/Allergan, Altimmune, Boehringer Ingelheim, Echosens, Fibronostics, Galecto, Gilead, Intercept, Madrigal, NorthSea, Novo Nordisk, Perspectum, Pfizer and Zydus. Research funding: 89Bio, Akero, AbbVie/Allergan, Altimmune, Better Therapeutics, Boehringer Ingelheim, Bristol-Myers Squibb, Corcept, DSM, Galectin, Genentech, Genfit, Gilead, Hepagene, Healio, Intercept, Inventiva, Ionis, Madrigal, Merck, NGM, Noom, NorthSea, Novo Nordisk, Perspectum, Pfizer, Poxel, Terns, Viking and Zydus. Honoraria: AbbVie/Allergan, Alexion, Echosens, Eisai, Exelixis, Gilead, Intercept, Perspectum, Salix, Siemens, Sonic Incytes and Theratechnologies.

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How to cite this article: Kugelmas M, Noureddin M, Gunn N, et al. The use of current knowledge and non-invasive testing modalities for predicting at-risk non-alcoholic steatohepatitis and assessing fibrosis. *Liver Int*. 2023;00:1-11. doi:10.1111/liv.15555