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NON-INVASIVE ASSESSMENT OF LIVER DISEASE IN PATIENTS WITH NAFLD

Laurent CASTERA (1), Mireen FRIEDRICH-RUST (2), Rohit LOOMBA (3)

(1) Department of Hepatology, Hôpital Beaujon, INSERM UMR 1149, University of
Paris-VII, Clichy, France

(2) Department of Internal Medicine 1, Division of Gastroenterology,
Hepatology, Goethe University Hospital, Frankfurt, Germany

(3) NAFLD Research center, Division of Gastroenterology, Department of
Medicine, University of California at San Diego, La Jolla, USA

Corresponding author: Prof. Laurent CASTERA, M.D., Ph.D.
Service d'Hépatologie, Hôpital Beaujon
Assistance Publique-Hôpitaux de Paris
100 boulevard du General Leclerc
92110 Clichy, France
e-mail: laurent.castera@bjn.aphp.fr

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is estimated to afflict approximately 1 billion individuals worldwide. In a subset of NAFLD patients, who have the progressive form of NAFLD termed as nonalcoholic steatohepatitis (NASH), it can progress to advanced fibrosis, cirrhosis, hepatocellular carcinoma and liver-related morbidity and mortality. NASH is typically characterized by a specific pattern on liver histology, including steatosis, lobular inflammation, ballooning with or without peri-sinusoidal fibrosis. Thus, key issues in NAFLD patients are the differentiation of NASH from simple steatosis and identification of advanced hepatic fibrosis. Until now, liver biopsy has been the gold standard for identifying these two critical endpoints but has well known limitations including invasiveness, rare but potentially life-threatening complications, poor acceptability, sampling variability and cost. Furthermore, due to the epidemic proportion of individuals with NAFLD worldwide, liver biopsy evaluation is impractical and non-invasive assessment for the diagnosis of NASH and fibrosis is needed. Although much of the work remains to be done in establishing cost-effective strategies for screening for NASH, advanced fibrosis and cirrhosis, in this review, we summarize the current state of the non-invasive assessment of liver disease in NAFLD, and we provide an expert synthesis of how these non-invasive tools could be utilized in clinical practice. Finally, we also list the key areas of research priorities in this area to move forward clinical practice.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD), affects around one **fourth** of the general population worldwide ¹. **Nonalcoholic steatohepatitis (NASH), the active form of NAFLD,** characterized by histological lobular inflammation and hepatocyte ballooning, **is associated with faster fibrosis progression and affects around 1.5% to 6.5% of the general population** ¹. NAFLD is frequently associated with metabolic comorbidities such as obesity (51%; 95% CI: 41-61), type 2 diabetes (22%; 95% CI: 18-28), hyperlipidemia (69%; 95% CI: 50-83), hypertension (39%; 95% CI: 33-46), and metabolic syndrome (42%; 95% CI: 30-56) ¹. Although the most common cause of death in patients with NAFLD is cardiovascular disease, independent of other metabolic comorbidities, NAFLD is becoming a major cause of liver disease-related morbidity (cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC) and liver transplantation) as well as mortality ^{2, 3}. **NAFLD is expected in the next decade to become the leading indication for liver transplantation in the USA** ⁴. It is estimated that liver-specific mortality and overall mortality among patients with NAFLD are 0.77 and 11.77 per 1,000 person-years whereas they are 15.44 and 25.56 per 1,000 person-years among patients with NASH ¹.

The vast majority of NAFLD patients however will not progress, only a minority, namely those with NASH and advanced hepatic fibrosis are at greatest risk of developing complications of chronic liver disease⁵. Indeed, advanced fibrosis has been shown to be the major driver for long-term outcome and mortality ⁶⁻⁸. Thus, key issues in patients with NAFLD are the differentiation of NASH from simple steatosis and identification of advanced hepatic fibrosis. Given the huge number of at-risk patients, there is a substantial unmet need for efficient and cost-effective means for risk stratification of NAFLD patients for these two critical endpoints. Liver biopsy, the gold

standard for identifying these two endpoints until now, appears unrealistic and unsuitable. In addition, it has well known limitations including invasiveness, poor acceptability, sampling variability and cost. As a result, this has fueled the development of alternative non-invasive strategies, which have been an area of intensive research over the past decade⁹.

This review is aimed at discussing the performance, advantages and limitations of non-invasive methods for the management of patients with NAFLD, including diagnosis and quantification of steatosis, differentiation of NASH from simple steatosis and identification of advanced hepatic fibrosis.

Currently available non-invasive methods and their limitations

Non-invasive methods rely on two different approaches: a “biological” approach based on the quantification of biomarkers in serum samples or a “physical” approach based on the measurement of liver stiffness, using either ultrasound- or magnetic resonance-based elastography techniques. Although these approaches are complementary, they are based on different rationales. Liver stiffness corresponds to a genuine and intrinsic physical property of liver parenchyma, whereas serum biomarkers indicate several, not strictly liver-specific clinical and serum parameters that have been associated with NASH or fibrosis stage, as assessed by liver biopsy.

Serum biomarkers

Current serum biomarkers (summarized in Table 1) include predictive models for diagnosing or grading steatosis (such as the Fatty Liver Index) or staging fibrosis (e.g NAFLD Fibrosis Score), direct **measures of hepatocellular damage** (e.g. circulating keratin 18 fragments) to differentiate patients with NASH from those with simple

steatosis and direct measures of fibrosis (e.g. PIIINP or Pro-C3) to discriminate patients with advanced fibrosis. Some are specific of NAFLD (e.g. BARD and NAFLD fibrosis scores) whereas some have been initially designed in hepatitis C (AST/ALT ratio, APRI, FIB-4). A few are proprietary formula (FibroTestTM, FibrometerTM, Hepascore and ELFTM score) but most are non-patented.

The practical advantages of analyzing serum biomarkers include their high applicability (>95%), their good inter-laboratory reproducibility, and their potential widespread availability (non-patented). However, none are liver specific—their results can be influenced by co-morbid conditions and they require critical interpretation of results.

Imaging techniques

Elastography

They are two different but complementary approaches for measuring liver stiffness: ultrasound-based and magnetic resonance-based elastography techniques. The first one uses ultrasound to detect the velocity of the microdisplacements (shear waves) induced in the liver tissue, whereas the latter uses the magnetic resonance scanner. The shear wave's velocity is then converted into a liver stiffness measurement, expressed in kilopascals (kPa) or in meters/second (m/s). Vibration-controlled transient elastography (TE) has been the pioneer ultrasound-based technique and is the most widely used worldwide but newer elastography modalities like point shear wave elastography, (pSWE), which includes Acoustic Radiation Force Impulse Imaging (ARFI), or two-dimensional shear wave elastography (2D-SWE), integrated in conventional ultrasound systems, are emerging¹⁰⁻¹². Their main characteristics, advantages and limitations are summarized in Table 2. TE and magnetic resonance elastography (MRE) provide

additional information in patients with NAFLD. The same machine can be used to determine whether steatosis is present : controlled attenuation parameter (CAP) for TE and calculation of the proton-density fat fraction (PDFF) for MRE. Comprehensive technical details can be found in the supplementary material.

Diagnosis and grading of Steatosis

Serum biomarkers

Several steatosis scores have been proposed for the detection of steatosis including the SteatoTestTM ¹³, the fatty liver index (FLI) ¹⁴, the hepatic steatosis index (HSI) ¹⁵, the lipid accumulation product (LAP) ¹⁶, the index of NASH (ION) ¹⁷ and the NAFLD liver fat score (NAFLD-LFS) ¹⁸. Their diagnostic performances have been summarized in a recent review ¹⁹. Although SteatoTestTM, FLI, NAFLD-LFS, LAP and HSI have been independently validated ²⁰⁻²³, their diagnostic performances are difficult to compare as they have been designed and validated against different standards: liver biopsy, ultrasonography, or magnetic resonance spectroscopy. Nevertheless, when FLI, NAFLD-LFS, HSI were retrospectively compared in the same cohort of 324 patients with suspected NAFLD and liver biopsy, their AUROC values for the diagnosis of steatosis (>5%) did not differ (0.83, 0.80 and 0.81, respectively) ²¹. Further studies are needed, but it should be acknowledged that these scores have not gained much popularity as they do not add much to the information provided by clinical, laboratory and imaging studies done routinely in patients with suspected NAFLD.

Ultrasonography

Conventional US is the most commonly used imaging method for the diagnosis of hepatic steatosis, since it is widely available, well established, well tolerated and cheap.

Typical US features are: hyperechogenicity as compared to the right kidney parenchyma, distal attenuation and the presence of areas of focal sparing²⁴. The degree of steatosis can be subjectively scored as mild, moderate and severe, or as reported in some studies by using ordinal US scores^{25,26}. **In a large meta-analysis²⁴ (n= 34 studies, 2815 patients with suspected or known liver diseases), pooled sensitivities and specificities of US to distinguish moderate-to-severe fatty liver from the absence of steatosis, taking liver biopsy as the reference, were 85% (80–89%) and 93% (87-97%), respectively.** However, in clinical practice mainly the presence or absence of steatosis is recorded and US has the limitation **that it can only detect steatosis with more than 12.5-20% liver fat content²⁷** and therefore, a relevant number of patients with steatosis starting at 5% liver fat content can be missed²⁸. In addition, the accuracy of US for diagnosis of liver steatosis is reduced in patients with obesity and coexistent renal disease^{29,30}. Recent studies obtained better results using quantitative ultrasound^{28,31}. Nevertheless, European guidelines for the management of NAFLD recommend to use ultrasound as first choice imaging in adults at risk for NAFLD².

Controlled Attenuation parameter (CAP)

In the initial study assessing its performances in 115 patients with chronic liver diseases (15% only with NAFLD), CAP was able to accurately detect steatosis $\geq 11\%$, $\geq 33\%$ and $\geq 66\%$ with AUROCs of 0.91, 0.95 and 0.89, respectively³². Nevertheless, despite a good correlation with histological steatosis, overlapped results between different grades of steatosis suggest that CAP cannot differentiate adjacent grades of steatosis with good precision. A recent individual data meta-analysis³³, based on 19 studies using the M probe and having included 2735 patients (537 with NAFLD; 19.6%), has reported for steatosis $\geq 11\%$, $\geq 33\%$ and $\geq 66\%$, AUROCs of 0.82, 0.86, and

0.88, respectively, sensitivities of 0.69, 0.77, and 0.88 and specificities of 0.82, 0.81 and 0.78, respectively. The authors proposed optimal cut-offs of 248 (237-261) dB/m, 268 (257-284) dB/m and 280 (268-294) dB/m, respectively. Interestingly, CAP values were influenced by several covariates including NAFLD, diabetes and BMI. Other authors, using MRI-PDFF as reference, have recently suggested 288 db/m as an optimal cut-off for detection of 5% or more fat in the liver³⁴. Table 4 summarizes the results of CAP in NAFLD patients³⁵⁻⁴⁵. Several comments can be made: most studies have been conducted in small sample size (< 100 patients) and heterogeneous populations with variable BMI and diabetes prevalence ; this may be an explanation for the differences in proposed cut-offs. However, the cut-off associated with significant steatosis (>33% of hepatocytes) is almost always >250 dB/m. Finally most of these studies have been performed with the M probe. In a recent US multicenter study, using the XL probe, in 393 NAFLD patients, CAP had an AUROC of 0.76 for detecting steatosis >5 % and a 96% positive predictive value at a cut-off of 263 dB/m⁴⁵. In contrast, the accuracy of CAP for separating steatosis $\geq 33\%$ and $\geq 66\%$ was suboptimal.

Thus far only two studies^{36, 46} compared head to head the performance of CAP with M and XL probes, using liver biopsy as reference, with conflicting results. In one study (236 Western patients with chronic liver disease with a mean BMI 24.4 \pm 6.3), the performances and cutoff values were similar⁴⁶, whereas in another study (57 NAFLD Chinese patients with a mean BMI 30.2 \pm 5.0), the performances were similar but cut-off values were higher with the XL probe³⁶. Therefore, further studies are necessary before any firm conclusions can be drawn.

Only two studies have performed a head-to head comparison of CAP with US, taking liver biopsy as reference: one in 72 patients with chronic liver disease⁴⁷ and the other one in 366 patients with chronic hepatitis B⁴⁸. Both studies showed that the

performance of CAP for detecting and grading liver steatosis was higher than that of US, however the rate of overestimation was significantly higher for CAP than for US (30.5% vs. 12.4%, $p < 0.05$)⁴⁸. More studies are needed before any firm conclusion can be drawn.

In the 3 studies^{39, 43, 44} comparing CAP and proton density fat fraction (PDFF) magnetic resonance (MR) spectroscopy for grading steatosis, using liver biopsy as reference, CAP was outperformed by MRI-PDFF. In a study in 78 American patients with NAFLD⁴³, MRI-PDFF performed better than CAP for diagnosing all grades of steatosis (AUROC 0.99 vs. 0.85, respectively, $p = 0.0091$). Similarly, in a study in 127 Japanese patients with NAFLD³⁹, MRI-PDFF had better diagnostic accuracy than CAP whatever the grade of steatosis. Finally, a study in 55 Dutch patients with NAFLD showed similar results⁴⁴.

Longitudinal studies are awaited. Recently, a study that followed up 4,282 patients who had both a reliable LSM and ≥ 10 successful CAP measurements has shown that neither the presence nor the severity of hepatic steatosis predicted liver-related events (LRE), cancer, or cardiovascular events in the short term while LSM, and etiology independently predicted LRE⁴⁹. Subgroup analyses of viral hepatitis (hepatitis B: 37.0%; hepatitis C: 2.9%) and NAFLD patients (40.7% of the entire cohort) revealed similar results.

In summary, CAP is a promising point of care technique for rapid and standardized steatosis quantification but needs to be better validated in patients with NAFLD with the XL probe. CAP is outperformed by MRI-PDFF but should to be compared to US that, despite its limitations, remains the most widely used tool for first line steatosis assessment.

MRI-PDFF

Cross-sectional utility of MRI-PDFF

MRS has been employed in several large epidemiologic studies and now with the development of MRI-PDFF it has been more widely utilized in epidemiologic studies to classify presence of hepatic steatosis as well as to quantify the amount of liver fat⁵⁰⁻⁵³.

Longitudinal comparison with histology and MRS

A series of single center studies suggested the utility of MRI-PDFF in the longitudinal assessment of changes in liver fat content with paired assessment with MRS and liver histology over a 24-week period⁵⁴⁻⁵⁶. These studies suggested that MRI-PDFF was more sensitive than liver histology in assessing changes in liver fat and has may be utilized in the setting of a clinical trial⁵⁷. These data have since been confirmed in multicenter studies in both adult as well as in pediatric population⁵⁸. These studies have shown that longitudinal change in MRI-PDFF robustly correlates with longitudinal change in MRS-PDFF (with correlation coefficients ranging from 0.96 to 0.99), when both the MRI and MRS measurements at each time points are meticulously co-localized^{55, 57}.

Role of MRI-PDFF as an endpoint in early phase NASH trial

Several early phase trials in NASH have adopted MRI-PDFF as an endpoint to examine efficacy of various drugs to assess treatment response. Le et al followed by the MOZART Trial proposed the need for co-localization of regions of interests (ROI) before and after treatment as the liver fat is heterogeneously distributed^{54, 55, 57}. MRI-PDFF is unable to assess liver inflammation, ballooning or resolution of NASH or improvement in fibrosis⁵⁹.

Clinical Utility of Amount of Decline in Liver Fat

As the new trial data emerged the experts started noticing a range of liver fat improvement in various trials. Using the paired MRI-PDFF and histology data from the MOZART trial it appeared that at a threshold of a relative 30% reduction in MRI-PDFF one may start to appreciate significantly higher odds of a 2 point improvement in NAFLD Activity Score on liver histology⁶⁰. These data require further validation that is ongoing in the multicenter setting⁶¹. Higher liver fat content at baseline in patients without fibrosis has been recently shown to be associated with significantly higher odds of fibrosis progression than those patients who have lower liver fat content⁶². These emerging data suggest that liver fat content may have prognostic significance, especially early-on the fibrosis progression cascade but need to be confirmed. MRI-PDFF estimation methods have been successfully implemented in the clinical setting as a tool for fat quantification. They are FDA approved and commercially available on the several MRI vendors including GE Healthcare, Siemens and Philips, and are now more readily available on newer scanners⁶³.

Diagnosis of NASH

Serum biomarkers

Many serum biomarkers have been investigated for the diagnosis of NASH⁶⁴ but cytokeatin (CK)-18 is by far the one that has been the most widely investigated. CK-18 fragments come from apoptosis of hepatocytes accomplished by the enzyme caspase 3 and can be measured in serum by immunoassay. **The M30 ELISA assay measures the caspase-cleaved K18 fragments and detects apoptosis, which is a hallmark of steatohepatitis, whereas the M65 ELISA assay detects total cell death.** Since the initial study by Feldstein et al.⁶⁵, reporting circulating serum levels of CK-18 to be predictive of NASH in patients with NAFLD (with AUROC of 0.83 and sensitivity of 0.75 and

specificity of 0.81 for a CK-18 value of about 250 U/L), many studies⁶⁶⁻⁷⁵ have confirmed these results, though in rather small populations. In two subsequent meta-analyses^{76, 77}, CK-18 had pooled AUROC of 0.82 (0.76-0.88) to predict NASH with a median sensitivity of 66%-78% and specificity of 82%-87%. There are however, several issues with CK-18 : lack of a commercially available clinical test⁷⁸, limited sensitivity at the individual level⁷⁹ and considerable variability in the suggested cut-offs and their respective diagnostic accuracy among studies that makes choosing which threshold to use very difficult⁶⁴. These limitations have resulted in limited clinical utility in practice so far. To increase CK-18 sensitivity, some authors have combined it with other biological parameters such as sFas levels⁸⁰, uric acid⁸¹, adiponectin and resistin (NASH diagnostics)^{74, 82}, or ALT and presence of metabolic syndrome (Nice Model)⁸³. Other predictive models, combining clinical and laboratory parameters, have been proposed for the diagnosis of NASH, including the HAIR (hypertension, increased ALT and Insulin Resistance)⁸⁴, the Palekar' score (age, sex, □ AST, BMI, □ AST/ALT ratio, □ and hyaluronic acid)⁸⁵, the Gholam score (AST and diabetes mellitus)⁸⁶, the oxNASH (13-hydroxyl-octadecadienoic acid/linoleic acid ratio, age, BMI and AST)⁸⁷, the NAFIC score (ferritin, insulin and Type IV collagen 7s)⁸⁸, and the NashTest™ (Biopredictive, Paris, France), a proprietary formula including twelve variables (age, sex, height, weight, serum levels of triglycerides, cholesterol, Alpha-2 macroglobulin, apolipoprotein A1, haptoglobin, GGT, aminotransferases ALT, AST, and □ total bilirubin)⁸⁹. In a meta-analysis from the developer, in 494 obese patients with a prevalence of NASH of 17.2%, the weighted AUROC of NashTest™ was 0.84²⁰. However, most of these models rely on small and highly selected populations (morbidly obese patients)^{83, 90-92} and have not been externally validated⁹³. The diagnostic performances of these different models have been recently reviewed and are therefore

not detailed in the present review^{78, 94}. Recently, several approaches using genetic biomarkers have been proposed including including single nucleotide polymorphisms (SNPs) located in *PNPLA3* such as the NASH Score (*PNPLA3* genotype, AST and fasting insulin)⁹⁵ and the NASH ClinLipMet Score (glutamate, isoleucine, glycine, lysophosphatidylcholine 16:0, phosphoethanolamine 40:6, AST, fasting insulin, and *PNPLA3* genotype)⁹⁶, but also expression of non-coding RNAs, specifically microRNAs (miRNAs) such as miR-122^{97, 98}. However, the information yielded has had moderate clinical utility.

In summary, none of the currently available serum marker are currently able to differentiate NASH from simple steatosis with high sensitivity and specificity, however, their diagnostic accuracy can be improved by combining different approaches.

Imaging techniques

Studies on the ability of elastography to discriminate between isolated steatosis and NASH are limited to TE and MRE. The performances of MRE have been investigated in 5 studies^{39, 43, 99-101} and those of TE in 4^{39, 43, 45, 102}. A wide range of AUROCS (0.35 to 0.93) and optimal cutoffs have been reported and likely depend on the prevalence of advanced fibrosis in the study population¹⁰³. In the 2 studies^{39, 43} with head to head comparison, there was no difference between TE and MRE. Thus currently, neither modality can reliably discriminate NASH from simple steatosis, although MR-based modalities are showing promise as discussed in the Other MR-Based Methods sub-section.

Staging of Liver Fibrosis

Serum biomarkers

The diagnostic performances of serum biomarkers have already been summarized in several reviews^{78, 94, 104} and therefore will not be detailed here. Briefly, as for **non-patented tests**, a recent meta-analysis (based on 64 studies in 13046 NAFLD patients) comparing BARD, APRI, FIB-4, and NFS for diagnosing advanced fibrosis reported summary AUROCS of 0.76, 0.77, 0.84, and 0.84, respectively¹⁰⁵. With an APRI threshold of 1.0 and 1.5, the sensitivities and specificities for advanced fibrosis were 50.0% and 84.0% and 18.3% and 96.1%, respectively. With a FIB-4 threshold of 2.67 and 3.25, the sensitivities and specificities for advanced fibrosis were 26.6% and 96.5% and 31.8% and 96.0%, respectively. The summary sensitivities and specificities of BARD score (threshold of 2), and NFS (threshold of 21.455) for advanced fibrosis were 0.76 and 0.61, 0.72 and 0.70, respectively. **Among the 4 biomarkers, FIB-4 and NFS are the most accurate with high negative predictive values (>90%) for ruling-out advanced fibrosis. They could therefore be used as first-line tools in primary health care setting to identify patients without advanced fibrosis who do not need further assesment. In that respect, FIB-4 may be more attractive to general practicionners, as it is based on widely available and simple parameters (age, transaminases and platelets) and easier to calculate than NFS. There are however several limitations that should be acknowledged: first, performances of FIB-4 and NFS to rule-in advanced fibrosis are rather inadequate meaning that further assesment with another test is needed in case of positive results (Figure 1). Second, it is important ot keep in mind that they have been mostly validated in liver clinics where the prevalence of advanced fibrosis is much higher than in primary health care settings ; third, when using FIB-4 or NFS, a significant proportion of patients (around 30%) fall in the intermediate risk category¹⁰⁶ (Figure 1) and cannot be correctly classified. This may lead to unecessary referral of these patients to liver clinics for further assesment. Finally, new age-adjusted cut-offs have been proposed recently to**

improve the diagnostic performance of NFS and FIB-4 for advanced fibrosis ¹⁰⁷.

As for patented tests, no independent meta-analysis is available. A recent French study ¹⁰⁸ from the developer, comparing Fibrometer® to other patented (FibroTest® and Hepascore) and non-patented (APRI, FIB-4, BARD and NFS) serum biomarkers in 452 NAFLD patients, showed that Fibrometer® (AUROC 0.82) outperformed all the other tests for diagnosing advanced fibrosis. These results require further independent confirmation. Finally, novel markers such as the PRO-C3, a commercially available assay that detects the synthesis of type III collagen, has been recently suggested to be superior to APRI, FIB-4 and NFS to identify patients with NAFLD and advanced fibrosis when combined with age, platelet and diabetes ¹⁰⁹. These promising results require however further validation. Finally, despite slight improvement in diagnostic accuracy over non-patented biomarkers, the limited availability of patented tests and their cost might limit their wider application.

In summary, among the different serum biomarkers studied, NFS and FIB-4 have been the most extensively studied and validated in different NAFLD populations and with consistent results. These tests perform best at excluding advanced fibrosis (with negative predictive values >90%) and could therefore be used as a first-line triage to identify patients at low risk of advanced fibrosis in settings where more sophisticated tests are unavailable ⁹.

Ultrasound-based Elastography

Transient elastography

Several meta-analysis, mostly performed in viral hepatitis patients, have reported good (88–89%) and excellent (93–96%), accuracies of TE for diagnosing advanced fibrosis and cirrhosis, respectively ¹¹. Two meta-analysis performed in NAFLD patients

have confirmed these results ^{76, 105}. The meta-analysis by Kwok, based on 9 studies (8 with the M probe) including a total of 1047 NAFLD patients, reported summary sensitivities of 85% and 92% and specificities of 82% and 92% for diagnosing advanced fibrosis and cirrhosis, respectively ⁷⁶. Table 3 summarizes the results of the most recent studies (since 2015) ^{39, 43, 108, 110-113} as prior studies have been already summarized ¹⁰⁴. These results deserve several comments : i), these studies have included heterogenous populations with a rather limited number of cirrhotic patients (<20%) and wide range of BMI (27 - 40 kg/m²); ii) the failure or unreliable results is lower when the XL probe is used. Also as shown in the most recent meta-analysis, based on 19 studies (4 using the XL probe) including a total of 2495 NAFLD patients from different ethnic backgrounds, summary AUROCs of TE did not differ between M and XL probes for diagnosing advanced fibrosis (0.87 vs. 0.86) and cirrhosis (0.92 vs. 0.94), respectively ¹⁰⁵ ; iii) apart from the type of probe used, the uneven distribution of fibrosis stages between studies may likely be an explanation for the observed differences between proposed cut-offs for a given endpoint, known as the spectrum bias ^{114, 115}. Finally, it should be stressed that all these studies have been conducted in tertiary referral centers where the proportion of patients with advanced fibrosis is higher than in the general population, thus making it difficult to extrapolate the performance of TE if used to detect cirrhosis in large populations. Overall, these results suggest that TE could be of interest to exclude confidently advanced fibrosis and cirrhosis with high negative predictive value (around 90%) in these patients ⁹. For instance, at a cut-off < 8 kPa, TE had a 94% to 100% negative predictive value ^{113, 116}. Finally, TE is recommended in the current guidelines on management of NAFLD ^{2, 9}.

Acoustic Radiation Force Imaging

Meta-analyses of point SWE using acoustic radiation force impulse (ARFI) imaging in patients with chronic liver disease reported diagnostic accuracies of 89–91% for advanced fibrosis and 92–93% for cirrhosis, with cut-offs ranging from 1.55–1.61 m/s for advanced fibrosis and 1.80–1.87 m/s for cirrhosis, respectively^{117, 118}. Other pSWE systems show comparable results to ARFI. However, different cut-offs are recommended for different systems^{10, 119}. Only few studies have evaluated pSWE using ARFI in patients with NAFLD with diagnostic accuracies of 84–98% for advanced fibrosis^{38, 110, 120–124}. A systematic review of 7 studies having included 723 NAFLD patients, reported a summary diagnostic accuracy, sensitivity and specificity of 90%, 80% and 85%, respectively for the detection of significant fibrosis¹²⁵. However, significant fibrosis is not the most relevant endpoint and no data are available to date for advanced fibrosis and cirrhosis. Similarly no data are available for the follow-up of NAFLD patients using pSWE. Therefore, pSWE is not included in the current guidelines on management of NAFLD.

Shear wave elastography

A retrospective meta-analysis, evaluating 2D-SWE in 1,340 patients with chronic liver diseases from 13 centres worldwide, reported diagnostic accuracies of 91% and 95% for advanced fibrosis and cirrhosis, and optimal cut-offs of 9.2, and 13.5 kPa, respectively¹²⁶. In the subgroup of 172 NAFLD patients, diagnostic accuracies were 93% and 92% for advanced fibrosis and cirrhosis, respectively, with the same optimal cut-offs as for the overall group. When 2D SWE was compared to TE in a subgroup of 91 NAFLD patients with reliable TE-values, 2D-SWE performed significantly better for diagnosing advanced fibrosis (AUROC difference of 12%, $p = 0.003$). In another study in 291 NAFLD patients, 2D-SWE had diagnostic accuracies of 89%, and 88% for

detecting advanced fibrosis and cirrhosis, respectively ¹¹⁰. The cut-off values with sensitivity above 90% were 8.3 kPa, and 10.5 kPa for advanced fibrosis and cirrhosis, respectively. Interestingly, 2D-SWE outperformed TE and ARFI only for significant fibrosis. No data are available for the follow-up of NAFLD patients using 2D-SWE. Therefore, 2D-SWE is not included in the current guidelines on management of NAFLD.

MR-elastography

2D-MRE has been shown in a prospective cohort of 117 patients with biopsy-proven NAFLD to have a high diagnostic accuracy for the detection of advanced fibrosis ¹⁰⁰. The AUROCs for the detection of any fibrosis, advanced fibrosis and cirrhosis were 0.84, 0.92 and 0.89, respectively, with an optimal cut-off for advanced fibrosis of 3.64 kPa. These results have been confirmed in a meta-analysis, based on 9 studies and 232 NAFLD patients ¹²⁷. In another recent meta-analysis, based on 5 studies and 628 NAFLD patients, the pooled AUROC of 2D-MRE for advanced fibrosis was 0.96 ¹⁰⁵.

In a head to head comparison between 3D MRE versus 2D MRE, 3D MRE at 40 Hz was superior to 2D MRE at 60 Hz with an AUROC for the detection for advanced fibrosis of 0.98 (3D-MRE) versus 0.92 (2D-MRE) ¹⁰¹. However, processing of 3D MRE takes a much longer time and has yet not been applied in multicenter studies. 3D MRE appears to be an extremely promising tool for longitudinal changes in fibrosis assessment. Further studies are needed to determine its role in fibrosis assessment in routine clinical practice.

Other MR-based methods:

A novel method termed as LiverMultiScan™ (Perspectum Diagnostics) has been recently proposed as a non-invasive, imaging-based biomarker to measure liver fat and correlate it with liver iron content, fibrosis and inflammation. The three parameters included in the proprietary algorithm are liver fat assessment, T2* and corrected T1 decay on advanced MRI¹²⁸. A pilot, proof-of-concept study has shown promising data, and further larger validation of these parameters in patients with biopsy-proven NAFLD, and its utility in assessment of treatment response in NASH trials is being actively assessed¹²⁸.

Recent novel data suggests that addition of damping ratio in addition to 2D-MRE and perhaps MRI-PDFP may help further advance the assessment of both inflammation and fibrotic components of disease activity and severity of NAFLD¹²⁹. Further studies are needed to examine the exact utility and applicability of these approaches in assessment of NAFLD severity.

Comparison and combination of approaches

Among clinical available modalities, MRE has the highest diagnostic accuracy in the detection of advanced fibrosis in NAFLD¹³⁰ but the evidence is based on a limited number of selected patients (<700) in highly specialized tertiary centers. In a head to head comparison with seven serum fibrosis markers, including FIB-4, in 102 patients with NAFLD, 2D-MRE performed better than all serum markers for the detection of advanced fibrosis¹³¹. When compared head to head with serum markers (FIB-4, NFS, APRI, BARD, Fibrometer® and FibroTest®) in large cohorts of NAFLD patients (452 and 761), TE outperformed all other serum markers¹¹⁶, apart from Fibrometer®¹⁰⁸. Some authors have proposed strategies combining TE with FIB-4 or NFS, either in a paired or in a serial fashion^{112, 116}. Such serial strategy however

increased the diagnostic performance with an accuracy around 70% but at the price of an uncertainty area (around 20%) and a 10% rate of misclassified patients¹¹⁶. MRE and TE have been compared head to head in patients with biopsy-proven NAFLD in three studies^{111 43 39} with conflicting results. Chen and colleagues have shown in 111 patients with morbid obesity (mean BMI 40.3 kg/m²) that MRE performed better than TE for detecting advanced fibrosis in intention to diagnose but not in per protocol analysis¹¹¹. In two other studies, one in 142 Japanese patients (mean BMI 28.1 kg/m²), using only the M probe³⁹ and one in 104 American patients (mean BMI 30.4 kg/m²), using both M and XL probes⁴³, there was no statistical difference between MRE and TE for the detection of advanced fibrosis. Differences in the studied populations might account for this discrepancy. Recent data suggest that, when staging fibrosis taking liver biopsy as reference, BMI is significantly associated with discordance of findings between MRE and TE, the degree of discordancy increasing with BMI¹³². **Finally, a recent individual patient meta-analysis (based on 230 patients) found that MRE had a statistically significantly higher diagnostic accuracy than VCTE in assessing each stage of fibrosis in patients with biopsy-proven NAFLD¹³³.** Therefore further studies are needed before any firm conclusion can be drawn. As for comparison with pSWE using ARFI, 2D MRE has been shown to be superior to pSWE for the detection of any fibrosis but not for advanced fibrosis¹²⁰. In addition, pSWE underperformed in the setting of obesity and higher liver fat content.

In summary, all of these modalities have a role in clinical practice and understanding the caveats associated with their utility (summarized in Table 2) are helpful in optimal clinical use of these tools.

Use in clinical practice

In patients with suspected NAFLD (presence of steatosis on ultrasound or abnormal liver tests (transaminases/GGT) in patients with risk factors such as obesity, type 2 diabetes or metabolic syndrome), non-invasive tests can be used in clinical practice for risk stratification. Whatever the approach, serum biomarkers or elastography, each modality is most reliable in excluding the presence of advanced fibrosis. As shown in Figure 1, the choice of non-invasive tools to be used should be guided by local availability and context of use. In primary health care setting, simple inexpensive and widely available serum biomarkers, such as FIB-4 or NAFLD Fibrosis scores, with high negative predictive value (> 90%) for ruling-out advanced fibrosis should be used as first-line. Patients with low risk (FIB-4 < 1.3 or NAFLD Fibrosis score < -1.455 ; 55% to 58% of cases) of having advanced fibrosis do not need further assessment. They should be offered lifestyle modifications and exercise. Those with intermediate (FIB-4 = 1.3 to 3.25 or NFS = -1.455 to 0.672 ; 30% of cases) and high risk (FIB-4 > 3.25 or NFS > 0.672 ; 12 to 15% of cases, positive predictive value 75-90%) should be addressed to a referral center for further assessment. Patented serum biomarkers (FibroTest®, Fibrometer® or ELF®) could be considered in patients with intermediate risk according to local availability. Otherwise TE, as the most widely available and best evaluated point-of-care technique, appears as the tool of choice, although ARFI and SWE are becoming increasingly available. XL probe should be used in patients with skin-liver capsule distance > 25mm in order to minimize the TE failure rate (<7%). Patients at low risk of having advanced fibrosis (LSM < 8 kPa ; NPV 94-100%) should be offered lifestyle modifications and reevaluation after 1 year. For those with intermediate (LSM = 8 to 10 kPa) or high risk (LSM ≥ 10 kPa, PPV 47-70%) of having advanced fibrosis should be considered for liver biopsy. However, confounders should be carefully excluded to minimize the risk of false positive. In case of TE failure despite

the use of XL probe or high BMI ($\geq 35 \text{ kg/m}^2$), alternative techniques such as MRE or SWE/ARFI may be considered according to local availability. However, although SWE and ARFI seem to be as promising as TE, data are currently limited for these modalities regarding the determination of advanced fibrosis in NAFLD. As for MRE, despite its high accuracy, cost and limited availability are limitations to its use in practice. Its role, as a surrogate of fibrosis improvement in therapeutic trials remains to be demonstrated. In any case, these patients should be offered lifestyle modifications and exercise and vitamin E (in non-diabetics) and pioglitazone may be considered as recommended by recent EASL or AASLD clinical practice guidelines ^{2, 3}. Finally, patients identified as having advanced fibrosis or cirrhosis should be screened for portal hypertension and liver cancer, given the increased risk of this disease in these individuals.

Special populations and controversies

Patients with type 2 diabetes mellitus are known to be at increased risk for NAFLD and advanced fibrosis. Non-invasive screening strategies for NAFLD, NASH or advanced fibrosis have been proposed in diabetic patients including the use of routinely available clinical variables ¹³⁴, TE ^{135, 136}, MRE ⁵² or combination of TE and ELF ¹³⁷. It is noteworthy that most studies on non-invasive tests in NAFLD patients have not been stratified for the presence of diabetes. Several recent studies suggested that non-invasive tests, which were developed and validated in non-diabetic cohorts, underperformed when applied to diabetic patients ¹³⁸⁻¹⁴⁰. Thus caution is requested when extrapolating results of non-invasive tests from non-diabetic populations to patients with diabetes. Also the role of ethnicity may be important to take into account ^{141, 142}, as most available studies have been done in Caucasians. Further studies are needed to address these issues.

Prognosis

Several recent studies have shown the ability of liver stiffness, measured using TE,¹⁰⁸ or serum biomarkers^{108, 143, 144} to predict clinical decompensation as well as survival in patients with NAFLD. A meta-analysis¹⁴⁵, based on 17 studies in 7058 patients with chronic liver diseases (mainly related to viral hepatitis), has shown that baseline liver stiffness, measured using TE, was associated significantly with risk of hepatic decompensation (6 studies; RR, 1.07; 95% CI, 1.03–1.11), hepatocellular carcinoma (9 studies; RR, 1.11; 95% CI, 1.05–1.18), death (5 studies; RR, 1.22; 95% CI, 1.05–1.43), or a composite of these outcomes (7 studies; RR, 1.32; 95% CI, 1.16–1.51). In a nationwide study (NAHNES cohort)¹⁴⁴ in 11,154 participants (34% with NAFLD) with a median follow-up of 14.5 years, those with a high probability of advanced fibrosis using APRI (>1.5), NFS (>0.676) or FIB-4 (>2.67), had a 69% increase in mortality compared to subjects without fibrosis (for NFS: HR, 1.69, 95% CI: 1.09-2.63; for APRI: HR, 1.85, 95% CI: 1.02-3.37; for FIB-4: HR, 1.66, 95% CI: 0.98-2.82) after adjustment for other known predictors of mortality.

Future directions

There is wealth of data that are informing the clinicians regarding the utility and limitations of each of the diagnostic modalities in the assessment of NAFLD. However, further advances are needed to refine clinical management and more accurate identification of patients at risk of fibrosis progression and those who need to be treated in the setting of a clinical trial without subjecting them to a liver biopsy evaluation. The key research priorities in the field are listed in Table 5. Addressing these gaps in knowledge would greatly impact the field.

Recently, efforts concentrating on “omics” approaches (lipidomics, proteomics and metabolomics) using high-throughput technologies have shown promising results to identify novel biomarkers of NAFLD, NASH and advanced fibrosis ¹⁴⁶. For instance, several studies based on lipidomics approaches have shown circulating oxidized fatty acids and products of arachidonic acid metabolism to be predictive of NASH ¹⁴⁷⁻¹⁴⁹. Similarly, proteomics have been used to identify NAFLD patients with active fibrosis, by measuring extracellular matrix remodeling rates in tissue and blood ¹⁵⁰. Using a metabolomic approach ¹⁵¹, subtypes of NAFLD with specific serum metabolomic profiles that differentiate steatosis from NASH in each subtype could be identified and might be used to monitor disease progression and identify therapeutic targets for patients. Finally, omics technologies have been used for the profiling of gut microbiota and identification of fecal-microbiome-derived metagenomic signatures associated with NASH and fibrosis in several human studies ^{152, 153}. It should be stressed however that these findings rely on small cross-sectional studies with a lack of external validation. In addition, the complicated methodology involved in omics platforms as well as reproducibility between centers and stability of samples and high cost prevent thus far widespread application in clinical practice.

Finally, given the high prevalence of NAFLD in the general population, non-invasive tests could be used as screening tools to identify patients with NAFLD at high risk of progression ¹⁵⁴. Recently, several studies have screened systematically for liver fibrosis, using either serum biomarkers ¹⁵⁵ or TE ¹⁵⁶⁻¹⁵⁸ the general population or at-risk populations ¹⁵⁹ or diabetics or those with a family history of NAFLD cirrhosis^{51 135}. Their results suggest an alarmingly high prevalence of chronic liver diseases, mainly related to NAFLD, ranging between 5% and 8% in the general adult population and between 18% and 27% among individuals with risk factors ¹⁶⁰. Thus, screening

programmes for liver fibrosis in the general population, using TE for identifying patients with presymptomatic chronic liver diseases susceptible to interventions should be further assessed.

Conclusions

Significant progress has been made regarding the non-invasive assessment of liver disease in patients with NAFLD. Use of non-invasive tests should be tailored according to the setting (primary health care, tertiary referral center, trial) and clinical needs (screening, staging of fibrosis, follow-up). Regarding detection and grading of steatosis, MRI-PDFF is the most accurate method but appears better suited for assessment and follow-up of selected patients in clinical trials whereas conventional ultrasound and if no steatosis is shown hereby CAP, as a point of care technique, could be used as triage in large unselected populations. Regarding NASH, no highly sensitive and specific blood tests are available to differentiate NASH from simple steatosis. Neither imaging modality can reliably discriminate NASH from simple steatosis, although MR-based modalities are showing promise. As for the identification of advanced fibrosis, MRE, TE, as well as FIB-4 and NFS are the most accurate and validated methods. FIB-4 and NFS are best suited as first-line tools in primary health care setting to confidently exclude advanced fibrosis whereas TE and MRE are more suited for referral centers to select the patients who require a liver biopsy. Finally, there is increasing evidence that serum markers and liver stiffness, measured using TE, accurately identify the subgroup of patients with NAFLD at a higher risk to reach the outcome of liver-related complications and death/liver transplantation.

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Figure 1 legend. A suggested algorithm for the use of non-invasive tests for risk stratification of patients with suspected NAFLD in clinical practice.

*Suspicion of NAFLD is based on the presence of steatosis on ultrasound or abnormal liver tests (transaminases/GGT) in patients with risk factors (Obesity, type 2 diabetes or metabolic syndrome). Significant alcohol consumption and secondary causes of steatosis should be excluded.

The proposed algorithm is based upon expert opinion. The choice of non-invasive tools should be **sequential**, guided by local availability and the context of use : in primary health care setting, simple inexpensive and widely available serum biomarkers, such as FIB-4 or NAFLD fibrosis score (NFS), with high negative predictive value (88-95%) for ruling-out advanced fibrosis should be used as first-line. Patients with low risk (FIB-4 < 1.3 or NAFLD Fibrosis score < -1.455 ; 55 to 58% of cases) do not need further assesment. **They should be offered lifestyle modifications and exercise.** Those with intermediate (FIB-4 = 1.3 to 3.25 or NFS = -1.455 to 0.672 ; 30% of cases) and high risk (FIB-4 > 3.25 or NFS > 0.672 ; 12 to 15% of cases, positive predictive value 75-90%) of having advanced fibrosis should be addressed to a referral center for liver stiffness measurement (LSM), using transient elastography (TE), in fasting condition, using M probe for patients with skin-liver capsule distance <25mm otherwise with the XL probe. Patients at low risk of having advanced fibrosis (LSM < 8 kPa ; NPV 94-100%) should be consider for a repeat evaluation within a year. For those with intermediate (LSM = 8 to 10 kPa) or high risk (LSM ≥ 10 kPa, PPV 47-70%) of having advanced fibrosis should be considered for liver biopsy. However, confounders for liver stiffness should be carefully excluded to minimize the risk of false positive results. ****Also patented serum biomarkers (FibroTest®, Fibrometer® or ELF®) could be considered in patients with intermediate risk according to local availability. In case of TE failure, alternative such as SWE/ARFI, MRE (particularly when BMI >35 kg/m²) may be considered according to local avaibility.** In any case, all patients should be offered lifestyle modifications and exercise. **As recommended by recent EASL or AASLD clinical practice guidelines, vitamin E (in non-diabetics) and pioglitazone may be considered in these patients. Also patients with cirrhosis should be screened for oesophageal varices and hepatocellular carcinoma.** In those with a liver biopsy, follow-up during treatment of LSM, using MRE, is the most promising non-invasive approach but requires further validation.

Abbreviations : NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; NPV, negative predictive value; PPV, positive predictive value; TE, transient elastography ; SWE shearwave elastography ; ARFI acoustic radiation force Imaging ; MRE, magnetic resonance elastography.

Table 1. Available serum biomarkers for diagnosing steatosis or for staging fibrosis in patients with NAFLD

Index (ref)	Items (n)	Age	Gender	BMI	Diabetes	Platelet count	AST level	ALT level	AST/ALT ratio	GGT level	TG level	Other components
Steatosis												
FLI ¹⁴	4			X						X	X	Waist circumference
HSI ¹⁵	3			X	X				X			-
SteatoTest® ¹³	12	X	X	X			X			X	X	A2M, ApoA1, haptoglobin, T bilirubin, cholesterol, and glucose
LAP ¹⁶	3		X								X	Waist circumference
ION ¹⁷	3/4		X					X			X	Waist-to-hip ratio (male yes ; female no), and HOMA
NAFLD-LFS ¹⁸	4				X				X			Metabolic syndrome and insulin

Fibrosis										
APRI ¹⁶¹	2					X		X		-
FIB-4 ¹⁶²	4	X				X		X	X	-
FibroTest® ¹⁶³	8	X	X	X					X	A2M, ApoA1, haptoglobin, and total bilirubin
Fibrometre NAFLD® ¹⁶⁴	7	X				X		X	X	glucose, ferritin and body weight
ELF® ¹⁶⁵	3									Hyaluronic acid, PIIINP and TIMP-1
Hepacore ¹⁶⁶	6	X	X						X	A2M, hyaluronic acid and total bilirubin
BARD score ¹⁶⁷	3			X	X				X	-
NFS ¹⁶⁸	6	X	X		X	X			X	Albumin

Abbreviation: FLI: fatty liver index ; HSI : hepatic steatosis index ; LAP : lipid accumulation product ; ION : index of NASH ; NAFLD-LFS :NAFLD liver fat score ; NFS : NAFLD fibrosis score ; A2M : alpha2-macroglobulin ; APOA1 : apolipoprotein A1.

Table 2. Respective characteristics, advantages and limitations of the 4 available elastography techniques for liver fibrosis staging

Techniques	Performed by	Units (range)	Steatosis grading	Quality criteria	Failure rate (%)	Confounders			Evidence in NAFLD Studies patients	Cost	Point-of-care
						Inflammation	Obesity	Others			
TE	Hepatologist trained nurse or technician	kPa (2-75)	Yes CAP	Well-defined IQR/M <30%	3-27	++	++ XL probe	Congestion Steatosis?	N=25 3862	\$	Yes
MRE	Radiologist	kPa* (2-11)	Yes PDFF	Emerging QIBA consensus statement	0-2	+	-	Congestion Iron overload	N=6 676	\$\$\$	No
pSWE/ARFI	Radiologist or ultrasonographer	m/sec (0.5-4.4)	No	Not well-defined	2	+ ? limited data	+ ? limited data	Similar to TE? limited data	N=8 834	\$\$	No
2D-SWE	Radiologist or ultrasonographer	kPa (2-150)	No	Not well-defined	13 ?	+ ? limited data	+ ? limited data	Similar to TE? limited data	N=2 447	\$\$	No

* MR elastography is reported as shear modulus, while US elastography techniques are reported in Young modulus. The Young modulus is three times the shear modulus.

Table 3. Performances of TE for the diagnosis of advanced fibrosis and cirrhosis in adult patients with NAFLD, taking liver biopsy as reference

Authors	Year	Design	Patients (n)	Age (yrs)	BMI (Kg/m ²)	Scoring system	Endpoint	Prevalence (%)	Cut-off (kPa)	AUC	Se (%)	Sp (%)	Failure or unreliable (%)	Probe
Petta	2015	R	179	45 ± 13	29.3 ± 4.1	Kleiner	F3-F4	23	7.9/9.6	0.86	85	81	13.0	M
et al. ¹¹²			142	44 ± 12	27.4 ± 3.7		F3-F4	20	7.9/9.6	0.85	68	86	23.0	M
Cassinotto et al. ¹¹⁰	2016	P	291	57 ± 12	32.1 ± 6.0	NASH-CRN	F3-F4	43	8.2/12.5	0.86	90/57	61/90	22.0	M
							F4	17	9.5/16.1	0.87	92/65	62/90		
Tapper	2016	P	164	51 ± 13	32.2*	Brunt	F3-F4	18	9.9	0.93	95	77	26.8	M
et al. ¹¹³							F4	NA						
Boursier et al. ¹⁰⁸	2016	R	452	56 ± 12	31.1 ± 5.2	NASH-CRN	F3-F4	38	8.7	0.83	88	63	14.1	M
							F4	13	-	0.87	-	-		
Imajo	2016	P	142	57 ± 15	28.1 ± 4.6	Brunt	F3-F4	32	11.4	0.88	86	84	11.0	M
et al. ³⁹			(10 C)				F4	8	14.0	0.92	100	76		
Petta	2017	R	324	54 ± 13	-	Kleiner	F3-F4	35	10.1	0.86	78	78	-	M
et al. ¹⁶⁹														
Park	2017	P	104	51 ± 15	30.4 ± 5.2	NASH-CRN	F3-F4	20	7.3	0.80	78	72	-	M
et al. ⁴³							F4	8	6.9	0.69	63	66	6.7	XL
Chen et al. ¹¹¹	2017	P	111	48*	40.3	Brunt	F3-F4	20	7.6	0.87	84	64	-	M
							F4	10	14.6	0.92	82	92	18.7	XL

Petta et al.	2017	R	761	59 ± 13	29.6 ± 4.9	Kleiner	F3-F4	31	7.9/9.6	0.86	90/74	65/81	-	M
Siddiqui et al. ⁴⁵	2018	P	393	51 ± 11	34.4 ± 6.4	NASH-CRN	F3-F4	32	8.6	0.83	80	74	-	M
							F4	9	13.1	0.93	89	86	5.0	XL

P prospective; R retrospective; C controls; Se, sensitivity; Sp, specificity. * median

Table 4. Performances of CAP for the diagnosis and grading of steatosis in patients with NAFLD, taking liver biopsy as reference

Authors	Year	Design	Patients (n)	Male gender (%)	Age (yrs)	Diabetes (%)	BMI (Kg/m ²)	Steatosis grading	Steatosis prevalence (%)	CAP Probe	Failure Rate (%)	AUC	CAP Cut-off (dB/m)	Se (%)	Sp (%)
Friedrich-Rust et al. ³⁸	2012	P	57	53	45 ± 14	-	28.0 ± 5.5	≥33%	74	M	19	0.78	245	97	67
								≥66%	46			0.72	301	76	68
Kumar et al. ⁴¹	2013	P	63	73	37*	-	25.1 ± 2.0	≥33%	59	M	-	0.79	258	78	73
								≥66%	11			0.77	283	71	68
Chan et al. ³⁵	2014	P	105	51	50 ± 11	52	29.4 ± 3.9	≥5%	97	M	4	0.97	263	92	94
								≥33%	64			0.86	281	97	68
								≥66%	14			0.75	283	100	53
Karlas et al. ⁴⁰	2014	P	50 (11 C)	50	55 ± 9	28	31.1 ± 4.2	≥5%	100	M	8	0.93	233	93	87
								≥33%	62			0.94	268	97	81
								≥66%	24			0.82	301	82	76
de Ledinghen et al. ³⁷	2016	R	261	59	56 ± 12	59	30.2 ± 5.1	>33%	72	M	24	0.80	310	79	71
								>66%	32			0.66	311	87	47
Imajo et al. ³⁹	2016	P	142 (10 C)	57	57 ± 15	50	28.1 ± 4.6	≥5%	83	M	11	0.88	236	82	91
								>33%	58			0.73	270	78	80
								>66%	17			0.70	302	64	74
Park et al. ⁴³	2017	P	104	43	51 ± 15	28	30.4 ± 5.2	≥5%	91	M	-	0.85	261	72	86
								>33%	43	XL	6.7	0.70	305	63	69

								>66%	15			0.73	312	64	70
Runge et al. ⁴⁴	2017	P	55	73	52*	-	27.8*	≥5%	91	M	0	0.77	260	90	60
								>33%	47			0.78	296	92	55
								>66%	16			0.78	334	78	76
Chan et al. ³⁶	2017	R	57	49	50 ± 10	-	30.2 ± 5.0	≥5%	98	M	6	0.94	260	91	87
			(22 C)					>33%	76	XL	2	0.80	266	91	87
								>66%	26			0.69	267	100	47
Naveau et al. ⁴²	2017	R	194	22	41 ± 01	18	44.0 ± 0.4	≥5%	85	XL	-	0.85	308	68	69
								>33%	59			0.59	335	65	79
								>66%	39			0.39	341	74	74
		P	123	26	40 ± 01	22	44.0 ± 0.6	≥5%	81			0.81	298	78	83
								>33%	58	XL	-	0.58	303	90	69
								>66%	37			0.37	326	83	71
Siddiqui	2018	P	393	32	51 ± 11	43	34.4 ± 6.4	≥5%	95	M		0.76	285	80	77
et al. ⁴⁵								>33%	57	XL	5.0	0.70	311	77	57
								>66%	27			0.58	306	80	40

P prospective; R retrospective; C controls; Se, sensitivity; Sp, specificity. * median

Table 5. Research priorities and unmet needs in the field

- Cut-point for each modality with the context of use needs to be determined (e.g. screening in primary care or screening in a diabetes clinic)
- Validation of quality criteria for each modality
- Cost-effectiveness of sequential use of clinical prediction rules (e.g. FIB-4) followed by TE/SWE/ARFI followed by MRE
- Clinically meaningful increase/decrease in liver stiffness that is linked to a clinical outcome in NAFLD
- Clinically meaningful increase in liver stiffness that is associated with a one stage increase in liver fibrosis
- Clinically meaningful decrease in liver stiffness that is associated with a one-stage decrease in liver fibrosis
- Cut-point for liver stiffness for each modality that is associated with a need to treat varices in patients with NAFLD
- Clinically meaningful decrease in liver stiffness that is linked to a clinical outcome in NAFLD
- Does reduction in liver stiffness in cirrhosis is associated with reduction in the risk of liver decompensation despite no change in fibrosis stage

Patients with suspected NAFLD*

1st line: primary health care

Search for other causes of liver disease (alcohol, HBV, HCV)

Rule-out/in advanced fibrosis
FIB-4 or NAFLD fibrosis score

FIB-4 < 1.3
NFS < -1.455

FIB-4 ≥ 1.3
NFS ≥ -1.455

Low risk

Intermediate to high risk

Attempt lifestyle modifications and exercise

No further assessment, repeat evaluation at 1 year?

2nd line: referral center

Rule-out/in advanced fibrosis
Transient elastography

LSM < 8 kPa

LSM ≥ 8 kPa

Low risk

Intermediate to high risk**

Consider liver biopsy

Failure (XL probe) 3.0%–6.7%

Consider MRE, 2D SWE, or ARFI according to local availability

Attempt lifestyle modifications and exercise
Vitamin E / pioglitazone?

Consider repeat evaluation (1 year)

Eligible for therapeutic trial? Monitoring with MRE?

Screen for OV and HCC if cirrhosis

