Non-invasive diagnosis and staging of non-alcoholic fatty liver disease

Stergios Kechagias¹ ² – Mattias Ekstedt¹ ² – Christian Simonsson³ ⁴ – Patrik Nasr¹ ²

Received: 11 March 2022 / Accepted: 19 May 2022 / Published online: 4 June 2022
© The Author(s) 2022

Abstract
Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of the metabolic syndrome and is characterized by ectopic accumulation of triglycerides in the cytoplasm of hepatocytes, i.e., steatosis. NAFLD has become the most common chronic liver disease, with an estimated global prevalence of 25%. Although the majority of NAFLD patients will never experience liver-related complications, the progressive potential of NAFLD is indisputable, with 5–10% of subjects progressing to cirrhosis, end-stage liver disease, or hepatocellular carcinoma. NAFLD patients with advanced fibrosis are at the highest risk of developing cardiovascular and cirrhosis-related complications. Liver biopsy has hitherto been considered the reference method for evaluation of hepatic steatosis and fibrosis stage. Given the limitations of biopsy for widescale screening, non-invasive tests (NITs) for assessment of steatosis and fibrosis stage, including serum-based algorithms and ultrasound- and magnetic resonance-based methods, will play an increasing role in the management of NAFLD patients. This comprehensive review presents the advantages and limitations of NITs for identification of steatosis and advanced fibrosis in NAFLD. The clinical implications of using NITs to identify and manage NAFLD patients are also discussed.

Keywords  Fibrosis · Non-alcoholic fatty liver disease · Non-invasive tests · Steatosis

Abbreviations
ALT    Alanine aminotransferase  FIB-4    Fibrosis-4
ARFI   Acoustic radiation force impulse  GCKR    Glucokinase regulatory protein
AST    Aspartate aminotransferase  GGT     Gamma glutamyltransferase
ATI    Attenuation imaging  HCC     Hepatocellular carcinoma
ATT    Attenuation coefficient  HDL     High-density lipoprotein
AUROC  Area under the receiver operating characteristic  ¹H-MRS  Proton magnetic resonance spectroscopy
BMI    Body mass index  HSD17B13 17β-Hydroxysteroid dehydrogenase 13
CAP    Controlled attenuation parameter  HSI     Hepatic steatosis index
CT     Computed tomography  HTGC    Hepatic triglyceride content
ELF    Enhanced Liver Fibrosis  HU      Hounsfield unit
ESLD   End-stage liver disease  IL-28B   Interleukin-28B
FLI    Fatty liver index  KLF6     Kruppel-like factor 6
FIB-4   Fibrosis-4
GCKR    Glucokinase regulatory protein
GGT     Gamma glutamyltransferase
HCC     Hepatocellular carcinoma
HDL     High-density lipoprotein
¹H-MRS  Proton magnetic resonance spectroscopy
HSD17B13 17β-Hydroxysteroid dehydrogenase 13
HSI     Hepatic steatosis index
HTGC    Hepatic triglyceride content
HU      Hounsfield unit
IL-28B  Interleukin-28B
KLF6    Kruppel-like factor 6
LAP     Lipid accumulation product
LSM     Liver stiffness measurement
MARC1   Mitochondrial amidoxime-reducing component 1
MAFLD   Metabolic dysfunction-associated fatty liver disease
MERTK   MER protocol-oncogene tyrosine kinase
MRE     Magnetic resonance elastography
MRI     Magnetic resonance imaging
MRI-PDFF Magnetic resonance imaging proton density fat fraction
NAFLD   Non-alcoholic fatty liver disease
NAFLD-LFS NAFLD liver fat score

© Springer
Introduction

Non-alcoholic fatty liver disease (NAFLD) affects approximately 25% of the global population [1] and has in recent years surpassed viral hepatitis as a major cause of chronic liver disease [2, 3]. NAFLD is the hepatic manifestation of the metabolic syndrome and encompasses a spectrum of histopathological features that range from ectopic accumulation of triglycerides in the cytoplasm of hepatocytes (i.e., steatosis) via establishment of inflammation and hepatocellular injury (i.e., non-alcoholic steatohepatitis (NASH)), to progressive fibrosis and risk of progression to cirrhosis and development of end-stage liver disease (ESLD) or hepatocellular carcinoma (HCC) [4, 5]. The presence of NAFLD is associated with type 2 diabetes mellitus (T2DM), as well as with a greater prevalence and incidence of cardiovascular disease and chronic kidney disease [6] (Fig. 1). In patients with T2DM, NAFLD prevalence ranges from 70 to 95%, while the rate is even higher in morbid obesity at up to 98% [1]. Subjects with T2DM are particularly susceptible to more severe forms of NAFLD and its associated consequences [7, 8] as they have a higher prevalence of advanced fibrosis compared to the general population [9]. The swift increase in the prevalence of NAFLD is also mirrored in the incidence of ESLD. In the USA, NAFLD is currently the second leading indication for liver transplantation [10] and the most rapidly growing cause of HCC among subjects listed for liver transplantation [11]. A modeling study suggests that by 2030, the prevalence of NASH will have risen by as much as 56%, with ESLD and mortality expected to more than double [12].

Liver biopsy is considered the reference method for diagnosing hepatic steatosis. Moreover, histologic evaluation of liver tissue enables assessment of the presence of NASH with or without advanced fibrosis. However, liver biopsy sampling represents only approximately 1/50,000 of the organ volume and has its own inherent limitations, such as cost, sampling errors due to inadequate sample acquisition, incorrect sample representation, observer variability, and risk of adverse events, making it unsuitable for large-scale screening [13]. Therefore, simple, easily accessible, and validated non-invasive tests (NITs) are of utmost importance and much needed in the management of NAFLD patients.

Presence of hepatic steatosis is the prerequisite for diagnosing NAFLD [14]. Therefore, this review will first focus on the non-invasive diagnosis of steatosis. Although the severity of steatosis is associated with development of T2DM and mortality [15], hepatic fibrosis has been shown to be the key independent predictor that is associated with all-cause, cardiovascular, and liver-related mortality in NAFLD patients [16–19]. Lobular inflammation and hepatocellular ballooning, included in the histopathological NAFLD activity score (NAS), have not been shown to predict progression of fibrosis or mortality [17, 18]. After the diagnosis of steatosis, the stage of fibrosis should be assessed appropriately. In clinical practice, identification of NAFLD patients with advanced fibrosis (F3-F4) is a priority as these patients are at higher risk of mortality and development of liver-related complications [20]. It is widely accepted that NAFLD patients with advanced fibrosis should be followed up in secondary care and should be prioritized for treatment once medications for NAFLD are recommended. Thus, this review will also focus on NITs to diagnose advanced fibrosis in NAFLD.

The exclusion of other liver diseases, including “significant” alcohol consumption, is necessary to establish a diagnosis of NAFLD. A panel of international experts recently suggested an alternative approach, i.e., “positive criteria” to diagnose the liver disease associated with the metabolic syndrome, while they also proposed the name metabolic dysfunction-associated fatty liver disease (MAFLD) [21]. The criteria are based on evidence of hepatic steatosis (detected either by imaging techniques, blood biomarkers/scores, or liver histology), in addition to one of the following three criteria, namely, overweight/obesity, presence of T2DM, or evidence of metabolic dysregulation. In contrast to NAFLD, MAFLD can coexist with other chronic liver diseases, for example, viral hepatitis or alcohol-related liver disease. To our knowledge, there are to date no large studies that have...
evaluated the performance of NITs in MAFLD using the proposed definition. Thus, it is currently unknown if the data presented in this review can be extrapolated to MAFLD.

Steatosis

Hepatic triglyceride content (HTGC) can be assessed using various methods that measure fundamentally different tissue properties. The conventional histopathological methodology used to quantify liver fat content consists of a visual semiquantitative approach in which the histopathologist uses a four-graded scale (0–3). Grades 0–3 are considered to correspond to fat deposition in <5%, 5–33%, 34–66%, and >66% of the hepatocytes, respectively [22, 23]. The diagnosis of NAFLD requires that ≥5% of the hepatocytes contain fat globules in the absence of overconsumption of alcohol and other (secondary) causes of steatosis. It has previously been demonstrated that semiquantitative assessment of steatosis by a histopathologist frequently overestimates hepatic fat content when measured quantitatively [24]. An alternative approach is to assess steatosis quantitatively with stereological point counting, a method with higher reproducibility suggested as being preferable when accurate histopathological measurements of hepatic steatosis are required [24]. Both the semiquantitative histological method and stereological point counting rely on biopsies and, as such, are invasive tests, which issue will not be further discussed in this review.

Serum-based steatosis biomarkers

Although levels of alanine aminotransferase (ALT) are the best single biochemical correlate of hepatic steatosis, serum levels of hepatic enzymes can be within reference limits in 50–79% of NAFLD patients, fluctuating or slightly elevated [25, 26]. There have been suggestions that the current upper limit of normal (ULN) for ALT may be too high and should be reduced significantly [27, 28]. Slight to moderate elevation of serum ferritin can be caused by dysmetabolic iron overload associated with steatosis. However, in many
NAFLD patients, elevated ferritin may reflect a subclinical inflammatory state rather than iron overload [29]. In general, a single biomarker cannot be used to identify NAFLD patients.

Several panels in which serum-based biomarkers are included have been evaluated as predictors of hepatic steatosis. Among them are fatty liver index (FLI) [30], hepatic steatosis index (HSI) [31], NAFLD liver fat score (NAFLD-LFS) [32], SteatoTest [33], visceral adiposity index (VAI) [34], triglyceride × glucose (TyG) index [35], and lipid accumulation product (LAP) [36, 37]. The diagnostic performance of these tests is summarized in Table 1. It should be noted that several of these tests have been evaluated using ultrasound as the gold standard to diagnose steatosis. However, ultrasound lacks sensitivity for detection of low grades of steatosis (see below) and, thus, the diagnostic performance of HSI, FLI, and LAP has probably been overestimated. In a study in which ultrasonography, SteatoTest, and liver biopsy were performed in 304 patients, it was shown that concordance between ultrasound and histopathologically was lower (kappa coefficient = 0.32 ± 0.05) than the concordance between SteatoTest and biopsy (kappa coefficient = 0.44 ± 0.06; P = 0.02) [33], indicating that both ultrasonography and serum-based tests have suboptimal sensitivity in detecting steatosis.

In a head-to-head comparison using liver biopsy as the reference standard, VAI outperformed four other algorithms with an area under the receiver operating characteristic (AUROC) of 0.92, a sensitivity of 79%, specificity of 92%, negative predictive value (NPV) of 16%, and positive predictive value (PPV) of 99% [38].

Given their diagnostic performance, the algorithms may have a role in identifying hepatic steatosis or cardiometabolic risk factors in population-based studies [36], but currently, they cannot be used in clinical practice for the diagnosis of NAFLD or in the decision-making process in the management of NAFLD patients. Further studies are needed to determine which algorithm(s) can be recommended as an initial tool for NAFLD screening. Moreover, it has not yet been clarified if these algorithms can be used for monitoring patients during interventions aiming to reduce liver fat.

### Table 1 Serum-based steatosis markers

| Blood markers/algorithms          | Components or formulas                                                                 | AUROC  | Study population (no. of participants)/ diagnostic tools |
|-----------------------------------|----------------------------------------------------------------------------------------|--------|---------------------------------------------------------|
| Hepatic steatosis index [31]      | $8 \times \text{ALT/AST} + \text{BMI} (+2, \text{if type 2 diabetes}; +2, \text{if female})$ | 0.72–0.82 | Korean ($n = 10,724$)/ultrasound                       |
| Fatty liver index [30]            | $(e^{0.953 \times \text{ln} (\text{TG}) + 0.139 \times \text{BMI} + 0.718 \times \text{ln (GGT)} + 0.053 \times \text{WC} - 15.745}) / (1 + e^{0.953 \times \text{ln} (\text{TG}) + 0.139 \times \text{BMI} + 0.718 \times \text{ln (GGT)} + 0.053 \times \text{WC} - 15.745}) \times 100$ | 0.79–0.85 | Italian ($n = 496$)/ultrasound                          |
| NAFLD liver fat score [32]        | $-2.89 + 1.18 \times \text{metabolic syndrome (yes = 1/\text{no} = 0) + 0.45 \times \text{type 2 diabetes (yes = 2/\text{no} = 0) + 0.15 \times \text{insulin (mU/L) + 0.04} \times \text{AST} - 0.94 \times \text{AST/ALT}$ | 0.78–0.87 | Finnish ($n = 470$)/$^1$H-MRS                         |
| SteatoTest [33]                   | $\text{ALT, } \alpha_2-\text{macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, GGT, total cholesterol, TG, glucose, age, gender, BMI}$ | 0.72–0.86 | Caucasians ($n = 2272$)/liver biopsy                  |
| Lipid accumulation product (LAP) [36, 37] | $[\text{WC (cm)} – 65 \text{ (male)} \text{ or } – 58 \text{ (female)}] \times [\text{TG (mmol/L)}]$ | 0.72–0.83 | NHANES III (development, $n = 9180$), Italian (validation, $n = 588$)/ultrasound (in evaluation study) Originally developed as an index of cardiometabolic risk |
| Visceral adiposity index [34]     | Male: $[\text{WC/39.68} + (1.88 \times \text{BMI})] \times [\text{TG/1.03}] \times (1.31/\text{HDL})$ | 0.92   | Validated in French NAFLD patients ($n = 324$)/liver biopsy Originally developed in patients with hepatitis C |
|                                   | Female: $[\text{WC/36.58} + (1.89 \times \text{BMI})] \times [\text{TG/0.81}] \times (1.32/\text{HDL})$ |         |                                                        |
| Triglyceride/glucose index [35]   | $\log (\text{TG} \times \text{glucose/2})$                                             | 0.90   | Validated in French NAFLD patients ($n = 324$)/liver biopsy Originally developed as a measure of insulin sensitivity |

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; BMI, body mass index; GGT, gamma glutamyltransferase; HDL, high-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; TG, triglycerides; WC, waist circumference
**Ultrasonography**

B-mode ultrasonography is widely used as the first-line imaging modality to detect hepatic steatosis due to its ease of use and low cost. This modality is the recommended screening method to detect steatosis in patients with T2DM by the European NAFLD guidelines [39]. Assessment of liver-to-kidney contrast, parenchymal brightness, deep beam attenuation, brightness of vessel walls, and gallbladder wall definition are used to diagnose excessive liver fat [40]. However, ultrasonography is hampered by limited sensitivity for detection of mild (<20%) steatosis [40]. Detection of steatosis may be improved by the combination of various echographic parameters during the examination and technical improvements in equipment, reaching a sensitivity of 80–85% at a liver fat content of ≥12.5% [41, 42]. However, in hepatic fat content of 5 to 9%, sensitivity can be as low as 12% [43]. Since the definition of NAFLD includes the presence of ≥5% hepatic steatosis on liver biopsy, this means that a considerable number of NAFLD patients with steatosis grade 1 on liver histology will not be identified by ultrasonography. Other limitations of ultrasonography include its inability to quantify liver fat and its subjectivity and examiner-dependent characteristics, which limit inter- and intraobserver reproducibility [44].

**Controlled attenuation parameter**

During the last few years, several techniques based on ultrasonography have been developed, which provide an improved assessment of liver fat, as compared to conventional B-mode ultrasonography, by implementing quantitative approaches. Among them are ultrasound-guided attenuation parameter (UGAP) [45], attenuation imaging (ATI) [46], attenuation coefficient (ATT) [47], and controlled attenuation parameter (CAP), which quantify liver fat by measuring the attenuation of radiofrequency and are based on the principle that echo attenuation is larger in liver tissue with any grade of hepatic steatosis than in the normal liver. The number of studies evaluating UGAP, ATI, and ATT is limited. CAP can be measured simultaneously with liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) and is widely used to assess hepatic steatosis [48]. The diagnostic accuracy of CAP has been validated extensively. A meta-analysis that compared histologically graded steatosis with CAP and included 2375 patients from 19 studies reported AUROCs of 0.82 with a CAP threshold of 248 dB/m for steatosis of >11%, 0.86 with 268 dB/m for steatosis of >33%, and 0.89 with 280 dB/m for steatosis of >66% [49]. However, the CAP value is affected by the presence of obesity and diabetes mellitus [48]. In NAFLD patients, the optimal thresholds for detecting magnetic resonance imaging proton density fat fraction (MRI-PDFF) of ≥5% and ≥10% were 288 dB/m and 306 dB/m, respectively, which were considerably higher than the optimal CAP thresholds obtained from a meta-analysis with multiple etiologies of liver disease [50]. In another meta-analysis including 2346 patients with chronic liver diseases, it was demonstrated that the accuracy of CAP in detecting histological liver fat >5% was fair (AUROC of 0.819) [51]. CAP has a high interobserver reproducibility of 0.82 [52], this feature giving it a clear advantage over conventional B-mode ultrasonography. One disadvantage though with CAP is its high rate of measurement failure (0–24%), particularly in obese patients [53]. To overcome this shortcoming, an obesity-specific probe (known as the XL probe) has been developed [54]. However, in NAFLD patients, the threshold for detecting steatosis seems to be higher with the XL probe compared with the conventional probe (known as the M probe) [55]. Thus, the optimal thresholds for detecting steatosis with the different probes remain to be clarified.

**Computed tomography**

The radiodensity of different tissues can be assessed with computed tomography (CT). The radiodensity of water is 0 Hounsfield units (HUs) by definition, and air is defined as −1,000 HUs [56]. In non-contrast CT, normal liver parenchyma is approximately 50 to 60 HUs, while fat is –20 to –100 HUs. Due to inconsistency in HU calibration by external factors, “fat-free spleen” can be used as an internal reference [56]. Hepatic HU <40 has been suggested as a cut-off value for steatosis (>30%) [57], while a liver HU–spleen HU value less than −9 to −10 can be used as a reference to detect steatosis [57–59]. However, as with ultrasonography, CT has limited sensitivity in detecting mild steatosis (<30% liver fat) and is also limited by radiation exposure [56, 57]. Thus, CT cannot be recommended as a primary diagnostic tool to detect steatosis.

**Magnetic resonance-based techniques**

Proton magnetic resonance spectroscopy (1H-MRS) is widely considered the most accurate non-invasive method with which to measure HTGC [60]. The technique identifies signals from protons associated with triglycerides by their resonance frequencies. The fat fraction is given as the fat signal divided by the sum of the water and fat signals [57]. An excellent correlation has been shown between 1H-MRS and total lipid quantification in specimens of liver tissue, and it has been suggested that 1H-MRS can replace liver biopsy for the assessment of liver fat content [61]. Compared to liver biopsy, a much larger volume (2 × 2 × 2 or 3 × 3 × 3 cm) of liver tissue is assessed, minimizing the likelihood of sampling error. However, 1H-MRS remains primarily a research
tool due to its low availability and limited clinical application [57, 62].

In contrast to MRS, magnetic resonance imaging (MRI) is available in many centers. MRI-PDFF is defined as the ratio of the mobile proton density from triglycerides and the total mobile proton density from triglycerides and water and reflects the concentration of triglycerides within liver tissue (Fig. 2). Although MRI-PDFF and histological assessment of fat content measure different properties, studies have demonstrated strong correlations between liver fat quantified by MRI-PDFF and steatosis grade as assessed by liver histology [43, 57]. Bannas et al. assessed the accuracy of MRI-PDFF through linear regression with 1H-MRS, triglyceride extraction, and histology using ex vivo human livers [63]. MRI-PDFF showed an excellent correlation with 1H-MRS ($r = 0.984$) and a strong correlation with histology ($r = 0.850$) and tissue triglyceride extraction ($r = 0.871$).

In a recent meta-analysis based on six studies including 635 patients with biopsy-proven NAFLD [64], the AUROC value of MRI-PDFF for detecting steatosis was 0.98. Pooled sensitivity and specificity were 93 and 94%. The diagnostic accuracy of MRI-PDFF has been compared with that of CAP in NAFLD patients. MRI-PDFF was superior when a liver biopsy was used as the reference standard [65, 66]. Moreover, in contrast to CAP, the diagnostic performance of MRI-PDFF to diagnose steatosis is not lower in NAFLD patients [64]. An additional advantage of MRI-PDFF is that, unlike ultrasound-based techniques, it can reliably detect longitudinal changes in hepatic triglyceride content as small as 2% or less [67].

In studies and clinical practice using 1H-MRS or MRI-PDFF to quantify liver fat content, a cut-off of 5% [68] or 5.56% is usually used to define hepatic steatosis. The latter value is based on the results of Szczepaniak et al. who examined the distribution of HTGC with 1H-MRS in 345 subjects with a low risk for hepatic steatosis, i.e., BMI < 25 kg/m², no glucose intolerance or excessive alcohol consumption, and normal serum ALT [62]. However, hepatic steatosis can be present in a significant proportion of lean subjects [69], as well as in individuals with normal serum ALT [70]. Nasr

Fig. 2 T2-weighted images (left) with schematic placement of 1H-MRS voxels ($3 \times 3 \times 3$ cm) placed in the right hepatic lobe and corresponding in vivo MRS spectra (right) for water (peak, 4.7 ppm) and triglycerides (peak, 1.3 ppm) for calculation of proton density fat fraction (PDFF) in a subject with a normal liver and in a patient with b NAFLD. Abbreviations: 1H-MRS, proton magnetic resonance spectroscopy; PDFF, proton density fat fraction; ppm, parts per million.
et al. [71] used histopathology as the reference standard to define the optimal cut-off for the definition of hepatic steatosis with 1H-MRS and reported that a cut-off of 3.02% could reliably identify patients with steatosis (specificity of 100%). A cut-off of 2% yielded the most reliable diagnostic accuracy, albeit with a few false positives (sensitivity of 87% and specificity of 94%). Thus, the cut-off for identifying NAFLD patients with MRI/MRS-based techniques can reliably be reduced to at least 3%. Other evidence also suggests that 5% as a limit for normal HTGC is too high. For example, it has been reported that a decrease in the suppression of endogenous glucose production is evident with HTGC as low as > 1.5% and that multiple extrahaepatic metabolic changes, such as muscle insulin resistance, hypertriglyceridemia, and decreased plasma high-density lipoprotein cholesterol, are fully established at HTGC 6% [25, 41, 72, 73].

An overview of imaging techniques to diagnose steatosis is given in Table 2.

### Fibrosis

Detecting fibrosis in NAFLD patients is critical as advanced fibrosis (F3-F4) independently predicts the development of liver-related complications, the need for liver transplantation, and liver-related and overall mortality [16–19]. Advanced fibrosis is also associated with a higher incidence of chronic kidney disease and increased mortality from cardiovascular disease [74]. Thus, there is a need to identify patients with bridging fibrosis (F3) and cirrhosis (F4) so that they can be managed to delay further progression, particularly given a large number of patients with undiagnosed cirrhosis within the general population (6–7%) [75].

Given the limitations of biopsy for widescale screening, NITs for assessment of fibrosis stage will play an increasing role in the management of NAFLD patients. The two main types of NITs used are the following: predictive models, which use clinical and laboratory data and imaging techniques, which estimate liver stiffness as a potential surrogate of hepatic fibrosis.

### Serum-based fibrosis biomarkers

The most common approach to assessing the stage of fibrosis by serological means consists of routine biochemical and/or hematological tests. These are indirect serum markers and are based on the evaluation of common functional alterations in the liver, alterations that do not necessarily reflect extracellular matrix turnover and/or fibrogenic cell changes. A better understanding of the pathophysiology of liver fibrosis has prompted investigators to use more refined markers to identify different fibrosis stages. These so-called direct serum markers are intended to detect extracellular matrix turnover and/or fibrogenic cell changes. Markers may be used alone or combined with patient and clinical characteristics or with other direct or indirect markers to form panels. An overview of the most common combinations of non-invasive serum-based biomarkers is presented in Table 3.

Fibrosis-4 (FIB-4) and NAFLD fibrosis score (NFS) are the most widely used algorithms and can be easily calculated using freely available online calculators [76, 77]. Both algorithms have high negative predictive values (approximately 90%) and can reliably exclude advanced fibrosis, thus identifying lower-risk patients who do not need secondary care referral. However, although FIB-4 and NFS are effective at excluding advanced fibrosis, as well as predictive of liver outcomes over time [78], they have limited ability to identify NAFLD patients with advanced fibrosis. Approximately 30% of NAFLD patients will be classified as “indeterminate” when using FIB-4 and NFS, and a significant number are falsely classified as having advanced fibrosis. Since there is abundant evidence that NFS and FIB-4 provide higher rates of false-positive results in older populations, it has been proposed that different diagnostic cut-offs should be used in patients ≥ 65 years old [79]. However, the latter proposal has been criticized since a recent study suggested that these adapted cut-offs significantly decreased the sensitivity to only 60% in patients over 65 years of age [80]. It should be noted that FIB-4 and NFS have been developed and, therefore, their coefficients for age have been calibrated in cohorts of patients aged around 45–50 years. Consequently, they are less sensitive in younger people and should not be used in patients < 35 years old [81]. This could become a matter of importance if cases of advanced liver fibrosis in young adults increase as expected due to the dramatic increase in the prevalence of obesity and insulin resistance in children [82].

Commercial biomarker panels such as the enhanced liver fibrosis (ELF) test, FibroTest (FibroSure), and FibroMeter have been developed, though they are more expensive and less available than FIB-4 and NFS. ELF is a simplified algorithm comprising three direct fibrosis markers which, aside from distinguishing patients with advanced fibrosis, may also be a good predictor of liver-related morbidity and mortality [82]. Therefore, this test may be considered in patients where FIB-4 and NFS indicate advanced fibrosis.

Several new scoring systems have also reported good accuracy in detecting advanced fibrosis in patients with NASH. Among them, an algorithm based on the measurement of serum PRO-C3 (a marker of type III collagen formation), age, presence of diabetes, and platelet count (ADAPT) [83], and the LINKI algorithms, which are based on hyaluronic acid, fasting glucose, AST, age, and platelet count [84]. In addition, the Hepamet fibrosis score has recently demonstrated superior diagnostic accuracy, compared to FIB-4 and NFS, in a multinational cohort of 1500 patients.
Table 2 Imaging techniques to diagnose steatosis

| Modality               | Description                                                                 | Diagnostic performance | Advantages                                                                 | Limitations                                                                 |
|------------------------|------------------------------------------------------------------------------|------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Ultrasonography        | - Tissue echogenicity depends on the degree of beam scattering               | AUROC 0.93 (Sn 60–80%, Sp 80–100%) | - Widely available                                                          | - Low sensitivity for mild steatosis                                     |
|                        | - Fat deposition in tissue accentuates scattering                           |                        | - Low cost                                                                  | - Operator-dependent                                                      |
|                        |                                                                              |                        | - No radiation                                                              | - Reduction of Sn and Sp in obese patients                                |
|                        |                                                                              |                        | - Easy to perform                                                           |                                                                            |
| Controlled attenuation  | - Measurement of the degree of ultrasound attenuation by hepatic fat        | AUROC 0.82 (Sn 69%, Sp 82%) | - Portable device                                                           | - High rate of measurement failure particularly in obese patients        |
| parameter (CAP)        |                                                                              |                        | - Immediate assessment of steatosis                                          |                                                                            |
|                        |                                                                              |                        | - Simultaneous liver stiffness measurement                                  |                                                                            |
| MRI-PDFF               | - Option that can be added to MRI scanners to quantitatively assess steatosis| AUROC 0.98 (Sn 93%, Sp 94%) | - Not affected by obesity                                                   | - Time-consuming                                                          |
|                        |                                                                              |                        | - Simultaneous MRI for liver architecture and focal lesions                | - Requires MRI facility                                                  |
|                        |                                                                              |                        |                                                                             | - Costly                                                                  |
|                        |                                                                              |                        |                                                                             | - Cannot be used in some patients with implantable devices               |
| $^1$H-MRS              | - Provides a collection of spectra for signal fat fraction estimation, which  | AUROC 0.98 (Sn 89%, Sp 92%) | - The absolute hepatic triglyceride concentration can be directly measured,| - See MRI-PDFF                                                            |
|                        | requires a proper acquisition technique to estimate hepatic triglyceride      |                        | and very small amounts (as low as 0.5%) can be detected and quantified     | - Complex and time-consuming data analysis                                |
|                        | content                                                                      |                        |                                                                             |                                                                            |

Abbreviations: AUROC, area under the receiver operating characteristic; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; $^1$H-MRS, proton magnetic resonance spectroscopy; Sn, sensitivity; Sp, specificity
Hormones (2022) 21:349–368

fibrosis [87].

dict the risk of incident severe liver outcomes in the general population and may be of value for detecting advanced liver outcomes [51].

FibroMeter[95] Platelets, prothrombin index, AST, α2-macroglobulin, hyaluronic acid, urea, age

BARD[89, 91, 92] BMI ≥ 28: No = 0, Yes = 1; AST/ALT ratio ≥ 0.8: No = 0, Yes = 2; and diabetes: No = 0, Yes = 1

ELF[93, 94] 2.2781 + 0.851 × ln [HA] (µg/L) + 10.751 × ln [P3NP] (µg/L) + 1.934 × ln [TIMP 1] (µg/L) + ln [ALT] (× 10^9/L) – 0.013 × platelet count (× 10^9/L) – 0.66 × albumin (g/dL)

FibroTest[96] Haptoglobin, α2-macroglobulin, apolipoprotein A1, GGT, bilirubin, age, gender

Table 3  Serum-based biomarkers of liver fibrosis in NAFLD

| Algorithm | Formula/parameters | Number of NAFLD patients | AUROC/Sn/Sp/NPV/PPV |
|-----------|-------------------|--------------------------|---------------------|
| APRI[88]  | (AST (U/L))/(AST upper limit of normal)/(platelet count (×10^9/L)×100) | 145 | 0.67/27/89/84/37 |
| FIB-4[88–90] | (age (years)×AST (U/L))/((platelet count (×10^9/L)×(ALT(U/L))^{2})) | 145 | 0.86/85/65/95/36 |
| NFS[88–90] | –1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet count (×10^9/L) – 0.66 × albumin (g/dL) | 733 | 0.82–0.88/77–82/71–77/88–93/52–56 |
| BARD[89, 91, 92] | BMI ≥ 28: No = 0, Yes = 1; AST/ALT ratio ≥ 0.8: No = 0, Yes = 2; and diabetes: No = 0, Yes = 1 | 827 | 0.81/–/–/–/– |
| ELF[93, 94] | 2.2781 + 0.851 × ln [HA] (µg/L) + 10.751 × ln [P3NP] (µg/L) + 1.934 × ln [TIMP 1] (µg/L) + ln [ALT] (× 10^9/L) – 0.013 × platelet count (× 10^9/L) – 0.66 × albumin (g/dL) | 61 | 0.87/89/96/96/80 |
| FibroMeter[95] | Platelets, prothrombin index, AST, α2-macroglobulin, hyaluronic acid, urea, age | 192 | 0.90/80/90/94/71 |
| FibroTest[96] | Haptoglobin, α2-macroglobulin, apolipoprotein A1, GGT, bilirubin, age, gender | 383 | 0.89*/81*/84*/77*/86* |

*Values are for prediction of significant fibrosis. ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; BMI, body mass index; DM, diabetes mellitus; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 score; GGT, gamma glutamyltransferase; IFG, impaired fasting glycemia; MMP, matrix metalloproteinases; NAFLD, non-alcoholic fatty liver disease; NFS, non-alcoholic fatty liver disease fibrosis score; NPV, negative predictive value; P3NP, N-terminal peptide of procollagen III; PPV, positive predictive value; TIMP, tissue inhibitors of metalloproteinases

[85, 86]. Recently, a Cox regression model based on age, AST/ALT-ratio, and ALT-level (dAAR) was reported to predict the risk of incident severe liver outcomes in the general population and may be of value for detecting advanced liver fibrosis [87].

Ultrasound-based methods

Techniques based on elastography measure tissue stiffness as a physical property termed Young’s modulus [97]. VCTE measures the speed of a mechanically induced shear wave through the hepatic parenchyma using pulse-echo ultrasonic acquisitions to obtain a liver stiffness measurement (LSM) as a marker of hepatic fibrosis. The velocity of the shear wave, which is perpendicular to the direction of pulse wave propagation, is proportional to liver stiffness, with quantitative results available as the algebraically derived Young’s modulus in kilopascals [97]. The result of VCTE is obtained as a median of at least 10 measurements and measures approximately a 1-cm diameter by 4-cm length region of liver tissue, which is approximately 100 times larger than that evaluated with liver biopsy [97]. VCTE is an easy-to-perform tool using a portable ultrasound probe, either an M-probe (3.5 MHz, at 2.5 to 6.5 cm depth) or, in the case of morbid obesity, an XL-probe (2.5 MHz, at 3.5 to 7.5 cm depth). Values of LSM < 8 kPa exclude advanced fibrosis with a very high probability [98]. However, the ability to rule in advanced fibrosis is considerably lower. Cut-offs ranging from 8 to 12 kPa have been proposed, with sensitivities ranging from 84 to 100% and specificities from 83 to 97% [48].

Obesity increases the probability of invalid results (by up to 30%) and may also lead to an overestimation of LSM [99, 100]. With the availability of the XL probe, which has been approved for use in patients with morbid obesity, the failure rate of VCTE was reported to be <5%. VCTE data are not reliable under conditions in which a rapidly developing mass effect inside the liver increases intrahepatic pressure and, thereby, reduces liver elasticity. This phenomenon is observed in right-sided congestive heart failure, acute inflammation and/or edema of the liver, and extrahepatic cholestasis [48]. In addition, regarding food intake, a minimum 2-h fast is currently recommended prior to the examination [48].

Shear wave elastography (SWE) and acoustic radiation force impulse (ARFI) are integrated into conventional ultrasonography devices to measure LSM [101]. Although SWE has been reported to perform better than ARFI in differentiating moderate fibrosis in NAFLD patients, VCTE, SWE, and ARFI showed similar performances in differentiating advanced fibrosis and suffered from a significant rate of failures or unreliable results [102].
Magnetic resonance-based methods

With magnetic resonance elastography (MRE), low-frequency vibrations are applied to the abdominal wall, which are tracked as propagating hepatic shear waves by acquiring images with wave motion-sensitized phase-contrast sequences and processing raw images (i.e., magnitude and phase images) to “wave images” and then to “elastogram” (Fig. 3). The cross-sectional elastogram images reflect the stiffness generated from the wave propagation information [103]. An MRE protocol can be performed with most MR scanners but requires adding hardware to generate mechanical waves and software for acquisition and processing. In theory, the whole liver can be examined, but typically four axial (or transverse) slices with a thickness of 5–10 mm are placed in the widest part of the liver, which generally corresponds to 5–35% of the total liver volume. Liver stiffness measurement is performed by drawing a region of interest on the generated elastograms, avoiding edge effects, large vessels, the gallbladder fossa, and any areas affected by cardiac and vascular artifacts. The mean stiffness is calculated using the mean from all regions of interest. Thus, MRE evaluates much larger volumes of the total liver than liver biopsy and can be performed in conjunction with conventional MRI.

Shear stiffness values in the normal liver are $< 3 \text{kPa}$ [103]. Studies have demonstrated that MRE has high diagnostic accuracy, with an AUROC of $\geq 0.90$ for identifying advanced fibrosis in NAFLD with MRE cut-off values ranging from 3.64 to 4.15 kPa [67, 104–106]. As with VCTE, MRE needs to be performed in the fasting state, at least for 2 h, because increased postprandial portal blood flow...
may cause a dynamic increase in liver stiffness, potentially leading to an overestimation of the extent of fibrosis [103]. In contrast to VCTE, diagnostic accuracy is not affected by patient body factors. In addition, the inter-observer agreement for staging fibrosis is nearly perfect and higher than that seen with histopathology [67, 105, 106].

The performance of MRE is better than that of VCTE-LSM in diagnosing advanced fibrosis in NAFLD patients [66]. However, unlike VCTE, it is difficult to implement MRE at a large scale since it requires an MRI facility, is associated with a high cost, and is time-consuming.

### Evolving non-invasive tests

#### Genetic markers

Genome-wide association studies have revealed several genetic risk factors associated with NAFLD prevalence and progression. Moreover, functional studies on those genes have added much knowledge on the pathogenesis of NAFLD and the complex pathophysiological pathways related to the disease [107]. The risk variant with the most pronounced effect on the development and progression of NAFLD is the rs738409 C > G single nucleotide polymorphism of patatin-like phospholipase domain-containing 3 (PNPLA3), which encodes the I148M protein variant of PNPLA3, also known as adiponutrin, a protein involved in lipid remodeling [108]. The primary hepatic effects of the I148M protein variant are most likely attributable to its repression of lipase activity [109], resulting in increased intracellular accumulation of triglycerides with the number of risk alleles (the G allele). In a recent study in which subjects were phenotyped by MRI-PDFF, each copy of the PNPLA3 risk allele was associated with a 2.56% increase in hepatic steatosis [110]. In addition, cumulative evidence also suggests that PNPLA3 is associated with fibrosis progression, development of HCC, ESLD, and all-cause mortality [111].

The gene encoding transmembrane 6 superfamily member 2 (TM6SF2) is also associated with NAFLD progression. The gene product of TM6SF2 is involved in VLDL secretion from hepatocytes, and the TM6SF2 E167K variant results in a loss of this function, resulting in the hepatic accumulation of lipids [112]. In NAFLD patients, TM6SF2 E167K is associated with significant fibrosis (stages 2–4), with an odds ratio (OR) of 1.88, independent of the PNPLA3 genotype [113].

Glucokinase regulatory protein (GCKR) is an inhibitor of glucokinase and regulates de novo lipogenesis [114]. The GCKRP446L variant disrupts negative regulation of glucokinase, which leads to glucose uptake and increased de novo lipogenesis. In a study comparing patients with NAFLD with healthy control individuals, significant associations were found between GCKR rs780094 and susceptibility to NAFLD (OR 1.49), NASH (OR, 1.55), and NASH with significant fibrosis (OR, 1.50) [115]. High levels of liver fat caused by these genetic mutations seem to be associated with disease progression in NAFLD. Thus, due to their influence on hepatic lipid accumulation, these genetic risk variants further support the hypothesis that high levels of liver fat are associated with the progression of NAFLD and probably other chronic liver diseases as well.

Interestingly, several genes protective of hepatic steatosis and fibrosis have recently been reported, namely, mitochondrial amidoxime-reducing component 1 (MARC1), 17β-hydroxyysteroid dehydrogenase 13 (HSD17B13), LPIN1, uncoupling protein 2 (UCP2), interleukin-28B (IL-28B), Kruppel-like factor 6 (KLF6), and the MER protocol-oncogene tyrosine kinase (MERTK) [111].

#### Omics-based markers

Through their ability to detect thousands of different molecules, “omics technologies” represent a new diagnostic approach that could be useful for the diagnosis of hepatic steatosis. A proteomics study conducted on 70 patients (35 controls and 35 NAFLD) identified 20 protein peaks in NAFLD versus controls (sensitivity of 89% and specificity of 83%). Moreover, it was reported that NAFLD patients had a higher basal hemoglobin level [116]. Metabolomics has been applied to identify the metabolic profile specific for NAFLD, such as glutathione and bile acids, whose levels are altered during NAFLD onset [117]. Lipidomic-based studies identify the alteration of lipid species levels, for instance, eicosanoids and short-chain fatty acids. It has been reported that three specific lipids (defined as “lipid triplet”) can identify NAFLD. However, the diagnostic performance was modest (AUROC of 0.71–0.74) [118]. Omics-based biomarkers may also be of value to evaluate disease severity. It was recently reported that a signature of triglycerides was able to differentiate steatosis from NASH, however, with limited diagnostic performance (AUROC of 0.79) [119].

#### RNA biomarkers

In biological fluids, there are RNA molecules belonging to different classes. A key feature making these RNAs potentially valuable biomarkers is their high stability since they are not present in free form in the circulation. Instead, they are encapsulated in membranous vesicles, complexed to RNA-binding proteins, or associated with lipoproteins, features that protect these RNAs from degradation [120]. Moreover, the techniques used for their detection are extremely sensitive. In theory, even a single RNA molecule could be detected through quantitative PCR [121].
Most of the studies concerning circulating RNAs as NAFLD biomarkers are limited to microRNAs (miRNAs). It has been reported that a miRNA panel (miR-122, miR-1290, miR-27b, miR-192) showed a high NAFLD diagnostic accuracy (AUROC of 0.86) [122]. Moreover, in a recent study, 2083 serum miRNAs were profiled in NAFLD patients representing the complete NAFLD spectrum and compared with population controls. The most robust finding was that serum miR-193a-5p levels correlated strongly with NAFLD activity grade and fibrosis stage. It was concluded that miR-193a-5p is a potential clinically tractable circulating biomarker for progressive NAFLD [123].

Gut microbiota

Several hypotheses have provided mechanistic insights into the pathways of how the gut microbiota might contribute to NAFLD development and progression [124]. Some studies have focused on microbiome signatures in NAFLD with fibrosis of different stages. When compared with NAFLD patients with severe fibrosis, individuals with absence of or mild fibrosis as well as healthy controls display a decreased abundance of Gram-negative bacteria, decreased Fusobacteria phylum, increased Enterobacteriaceae family (Bacteroides, Ruminococcus, and Shigella genera [125, 126]), and, by contrast, increased Gram-positive bacteria, Firmicutes phylum, Prevotellaceae family, and Prevotella genus [127].

In a recent study, Lang et al. used gut microbiota-based approaches, VCTE, NFS, and FIB-4, to predict advanced fibrosis in 83 biopsy-proven NAFLD patients. A random forest model composed of clinical features and bacterial taxa achieved an AUROC of 0.87, which was similar to the AUROCs of NFS and FIB-4 (0.86 and 0.85, respectively). VCTE had the best diagnostic performance with an AUROC of 0.93 [128].

Clinical implications

Which patient populations should be screened for NAFLD?

Current guidelines do not recommend widespread or community screening, mainly due to the perceived associated direct and indirect medical costs [20, 39]. However, some national and international initiatives (ETHON project, Spain [129]; international LiverScreen project [NCT03789825]) are currently investigating the effectiveness of screening the general population for significant liver disease. Screening at-risk patients has been shown to be cost-effective in several studies across different countries [130–132]. To define at-risk populations, important information can be obtained from population-based studies evaluating the screening for liver fibrosis using VCTE [81, 133–135]. Using the 8.0 kPa threshold, the prevalence of patients at risk of significant liver fibrosis among adults in the general population was similar across studies (6–7%). However, in some subgroups, the prevalence was significantly higher. T2DM has been shown to be the condition associated with the highest risk of increased LSM [133]. The prevalence of liver stiffness ≥ 8.0 kPa was approximately 9% in patients with diabetes but no liver steatosis, and it reached 17.2% in patients with both diabetes and liver steatosis [134].

Other factors associated with elevated liver stiffness are closely related to metabolic conditions such as obesity, impaired fasting glucose, low HDL-cholesterol, and high triglyceride levels. [8, 136–138]. The available evidence, therefore, suggests that patients with BMI > 25 kg/m², particularly those with metabolic risk factors, and especially T2DM, might be a relevant population for the identification of NAFLD patients with advanced fibrosis. However, it is unclear at which age at-risk patients should be screened. Most studies have been performed in adults aged > 40 years, although obesity and T2DM have become more prevalent in children. Moreover, T2DM tends to be more aggressive in young people with poorer responses to glucose-lowering medication and greater insulin resistance [139–141]. As the length of exposure to insulin resistance is probably a key factor in the development of NAFLD with advanced fibrosis, it is reasonable to assume that more cases of advanced NAFLD will be identified in young adults in the near future. In this context, it was recently reported that the prevalence of elevated LSM (≥ 7.9 kPa) in young adults aged 22–25 years was 2.7% [81].

Which method should be used to identify steatosis?

As stated above, 1H-MRS and MRI-PDFF can be regarded as reference methods to diagnose steatosis. These methods provide a more accurate assessment of steatosis than conventional histopathology, which often overestimates hepatic triglyceride content [24, 71]. However, despite the high accuracy of MRI-PDFF for detecting and quantifying steatosis, cost and limited availability restrict its use on a larger scale.

Serum-based algorithms to identify steatosis have been proposed for screening purposes, but, in general, their diagnostic performance is suboptimal compared to other methods, and currently, they do not add much to the information provided by the clinical, laboratory, and imaging examinations that are routinely performed in patients with suspected NAFLD.

Conventional ultrasonography is currently recommended as the first-line tool for the diagnosis of steatosis in clinical practice [39]. It is widely available, cheap, and well-established. However, ultrasonography has poor sensitivity for detecting steatosis < 20% and many NAFLD patients may...
thus be missed when using this modality. CAP is a promising technique for the detection of steatosis, but it is unknown whether its diagnostic accuracy is better than that of ultrasonography in patients with steatosis < 20%. If NAFLD patients with steatosis missed by ultrasound-based techniques have a low risk of developing advanced fibrosis, the inability of conventional ultrasonography and CAP to identify steatosis < 20% would be of limited clinical importance. However, a phenomenon exists in NAFLD known as burned-out NASH, in which steatosis decreases with the progression of fibrosis [142]. In a study including 458 NAFLD patients with advanced fibrosis, overall mortality was higher in patients with histological steatosis < 33% than in those with steatosis ≥ 33% [143]. Thus, some NAFLD patients with a low steatosis grade not identified with ultrasound-based methods might have advanced fibrosis and an increased risk of liver-related events and mortality. The characteristics of NAFLD patients diagnosed with MRI-PDFF but missed by ultrasound-based techniques will be elucidated in the currently ongoing prospective Swedish EPSONIP study [144] in which patients with T2DM consecutively enrolled from primary care health centers will undergo serological, ultrasound-based, and magnetic resonance-based repeated measurements of steatosis and fibrosis.

**Which method should be used to identify advanced fibrosis in NAFLD?**

The most validated NITs for assessment of fibrosis stage in NAFLD are NFS and FIB-4, which are non-patented and widely available tests. FIB-4 is easier to calculate since it is composed of only three serum-based parameters and the subject’s age, and thus may be most suitable for screening purposes. Both algorithms can exclude the presence of advanced fibrosis with a high NPV (> 90%) [145]. However, their PPV for confirming advanced fibrosis is modest (< 70%), with a significant risk of false-positive results [145]. Moreover, approximately one-third of patients fall in between the lower and upper cut-offs, resulting in undetermined results [145]. The insufficient PPV of these tests has led to the development of strategies using FIB-4 or NFS as a first-line procedure, followed, if positive, by a second-line confirmatory test (elastography or specialized blood test). A proposed pathway to assess the fibrosis stage in NAFLD patients is shown in Fig. 4.

The two most validated patented serum fibrosis biomarkers are FibroMeter and ELF. Overall, the diagnostic accuracy of patented serum fibrosis tests for staging fibrosis is similar [146] to that of FIB-4 and NFS or only slightly higher [147]. However, high costs and limited availability limit the widespread application of these tests in clinical practice.

VCTE is the most widely available device for LSM with the largest amount of data in the NAFLD setting. It has significantly better diagnostic accuracy compared to FIB-4 and NFS [145] and is, therefore, better suited as a second-line test. There is no agreement in clinical practice on LSM cut-offs for ruling out advanced fibrosis, even though 8 kPa is the most validated threshold, with an NPV > 90%. However, the PPV to confirm advanced fibrosis is not sufficiently high to avoid liver biopsies in many NAFLD patients. According to the results of a recent meta-analysis [98], values of LSM by VCTE > 12 kPa could be used to rule in advanced fibrosis, thus limiting the need for confirmatory liver biopsy in NAFLD patients with LSM values by VCTE between 8 and 12 kPa, albeit this must be confirmed in further studies. Regarding SWE, meta-analyses [148, 149] suggest performance in detecting advanced fibrosis similar to that reported for VCTE [102]. However, SWE is less available and relevant data in NAFLD patients remain limited.

MRE has superior diagnostic accuracy compared to ultrasound-based elastography techniques. However, the use of MRE is limited to tertiary specialized centers because of its high cost and the need for advanced equipment.

**Should screening aim to identify steatosis, advanced fibrosis, or earlier stages of fibrosis?**

In light of the pathophysiology of NAFLD, which implies the initial accumulation of triglycerides leading to steatosis and, in some individuals, subsequent progressive fibrosis, it is reasonable to screen for steatosis in patients with metabolic risk factors such as T2DM and hyperlipidemia, or with BMI > 25 kg/m². Considering that high levels of liver fat appear to be associated with the progression of NAFLD, ultrasonography, which is widely available and has sufficient sensitivity to identify pronounced steatosis, is currently the screening method of choice. Further investigations can be avoided in subjects without steatosis, while in those with steatosis, an NIT, preferably FIB-4, should be used to assess the probability of advanced fibrosis (F3-F4). If positive, a second-line confirmatory test (elastography or specialized blood test) should be conducted.

However, applying the strategy above theoretically entails a risk of missing advanced fibrosis in burned-out NASH or in NAFLD patients with low steatosis grade not detectable with ultrasonography. Therefore, an alternative screening approach may be to disregard steatosis and focus on screening for advanced fibrosis with serum-based NITs and/or elastography in populations at risk for NAFLD. Future studies in subjects with metabolic risk factors assessing the prevalence of advanced fibrosis with normal HTGC or with low steatosis grade undetectable with ultrasonography, as well as the diagnostic performance of NITs in these patients, will elucidate whether this strategy may be preferable.

Identifying earlier stages of fibrosis in NAFLD is appealing since this would give physicians opportunities to
intervene at an earlier stage, minimizing the risk of future liver-related complications. However, considering the inferior diagnostic performance of NITs to distinguish fibrosis stages 1–2 and in light of the absence of approved pharmacological treatments to halt or retard disease progression, it is currently advisable to focus on identifying NAFLD patients with advanced fibrosis. The presence of advanced fibrosis, particularly cirrhosis, alters clinical management, especially the initiation of surveillance for gastroesophageal varices and HCC.

Conclusions

The increasing prevalence of obesity has changed the landscape of chronic liver disease, with increasing numbers of NAFLD patients presenting with complications of ESLD. In many of these patients, NAFLD and its severity have previously been undiagnosed or neglected, and opportunities for early interventions and surveillance have been missed.

Accumulating evidence supports the use of NITs for diagnosing steatosis and assessing the presence of advanced fibrosis in NAFLD. The diagnostic performance of serum-based algorithms is currently insufficient for clinical use to identify patients with steatosis. CAP and other ultrasound-based methods are promising, but diagnostic cut-offs for steatosis are yet to be defined. $^1$H-MRS or MRI-PDFF can reliably diagnose steatosis and replace liver biopsy as the reference method in this aspect. On the other hand, their limited availability limits the implementation of these methods on a large scale. Serum-based algorithms are recommended as the first step to assess the fibrosis stage in NAFLD. If advanced fibrosis cannot be excluded with these algorithms, VCTE offers higher diagnostic accuracy with excellent NPV but with considerably lower PPV. Thus, liver biopsy may still be needed to assess the fibrosis stage in a significant number of NAFLD patients. MRE may reduce the need for liver biopsy, although further studies are needed to clarify its usefulness in the evaluation and follow-up of NAFLD patients. Genetic, omics-based, and RNA markers, as well
as gut microbiota-based approaches are promising methods, but their role in the management of NAFLD patients remains to be clarified.

Funding Open access funding provided by Linköping University.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M (2016) Global epidemiology of nonalcoholic fatty liver disease - meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64:73–84. https://doi.org/10.1002/hep.28431

2. Marcellin P, Kutala BK (2018) Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. Liver Int 38(Suppl 1):2–6, https://doi.org/10.1111/liv.13682

3. Younossi ZM (2019) Non-alcoholic fatty liver disease - a global public health perspective. J Hepatol 70:531–544. https://doi.org/10.1016/j.jhep.2018.10.033

4. Ekstedt M, Fransen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G et al (2006) Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 44:865–873. https://doi.org/10.1002/hep.21327

5. Nasr P, Ignatova S, Kechagias S, Ekstedt M (2017) Natural history of nonalcoholic fatty liver disease: a prospective follow-up study with serial biopsies. Hepatob Commun 2:199–210. https://doi.org/10.1002/hep4.1134

6. Targher G, Tilg H, Byrne CD (2021) Non-alcoholic fatty liver disease: a multisystem disease requiring a multidisciplinary and holistic approach. Lancet Gastroenterol Hepatol 6:578–588. https://doi.org/10.1016/S2468-1253(21)00020-0

7. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zeneri L et al (2007) Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 30:1212–1218. https://doi.org/10.2337/dc06-2247

8. Kwok R, Choi KC, Wong GL, Zhang Y, Lik-Yuen Chan H, On-Yan Luk A et al (2016) Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut 65:1359–1368. https://doi.org/10.1136/gutjnl-2015-309265

9. Bril F, Cusi K (2017) Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: a call to action. Diabetes Care 40:419–430. https://doi.org/10.2337/dc16-1787

10. Nourreddin M, Vipani A, Breeze C, Todo T, Kim IK, Alkhouri N et al (2018) NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. Am J Gastroenterol 113:1649–1659. https://doi.org/10.1038/s41395-018-0088-6

11. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A et al (2019) Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. Clin Gastroenterol Hepatol 17:748–755. https://doi.org/10.1016/j.cgh.2018.05.057

12. Estes C, Ansee QM, Arias-Losle MT, Bantel H, Bellentani S, Caballeria J et al (2018) Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. J Hepatol 69:896–904. https://doi.org/10.1016/j.jhep.2018.05.036

13. Sumida Y, Nakajima A, Itoh Y (2014) Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 20:475–485. https://doi.org/10.3748/wjg.v20.i2.475

14. Adams LA, Angulo P, Lindor KD (2005) Nonalcoholic fatty liver disease. CMAJ 172:899–905. https://doi.org/10.1503/cmaj.045232

15. Nasr P, Fredriksson M, Ekstedt M, Kechagias S (2020) The amount of liver fat predicts mortality and development of type 2 diabetes mellitus in non-alcoholic fatty liver disease. Liver Int 40:1069–1078. https://doi.org/10.1111/liv.14414

16. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM et al (2020) Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology 158:1611–1625. https://doi.org/10.1053/j.gastro.2020.01.043

17. Ekstedt M, Hagström H, Nasr P, Fredriksson M, Stål P, Kechagias S et al (2015) Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 61:1547–1554. https://doi.org/10.1002/hep.27368

18. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Björnsson ES, Charatcharoenwitthaya P et al (2015) Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 148:389–397. https://doi.org/10.1053/j.gastro.2015.04.043

19. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R et al (2017) Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 67:1265–1273. https://doi.org/10.1016/j.jhep.2017.07.027

20. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi C, Rinella M et al (2018) The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 67:328–357. https://doi.org/10.1002/hep.29367

21. Eslam M, Newsome PN, Sarin SK, Ansee QM, Targher G, Romero-Gomez M et al (2020) A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 73:202–209. https://doi.org/10.1016/j.jhep.2020.03.039

22. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474. https://doi.org/10.1111/j.1572-0241.1999.01377.x

23. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al (2005) Design and validation of a
histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313–1321. https://doi.org/10.1002/hep.20701

24. Franzen LE, Ekstedt M, Kechagias S, Bodin L (2005) Semi-quantitative evaluation overestimates the degree of steatosis in liver biopsies: a comparison to stereological point counting. Mod Pathol 18:912–916. https://doi.org/10.1038/modpathol.3800370

25. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC et al (2004) Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40:1387–1395. https://doi.org/10.1002/hep.20466

26. Bhatia LS, Curzen NP, Calder FC, Byrne CD (2012) Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? Eur Heart J 33:1190–1200. https://doi.org/10.1093/eurheartj/eur453

27. Kunde SS, Lazenby AJ, Clemens RH, Abrams GA (2005) Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. Hepatology 42:650–656. https://doi.org/10.1002/hep.20818

28. Prati D, Collli A, Conte D, Colombo M (2005) Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. Hepatology 42:1460–1461. https://doi.org/10.1002/hep.20964

29. Kechagias S, Nasr P, Blomdahl J, Ekstedt M (2020) Established and emerging factors affecting the progression of nonalcoholic fatty liver disease. Metabolism 111S:154183. https://doi.org/10.1016/j.metabol.2020.154183

30. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A et al (2006) The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 6:33. https://doi.org/10.1186/1471-230X-6-33

31. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W et al (2010) Hepatic steatosis index: a simple screening tool reflecting non-alcoholic fatty liver disease. Dig Liver Dis 42:503–508. https://doi.org/10.1016/j.dld.2009.08.002

32. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM et al (2009) Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 137:865–872. https://doi.org/10.1053/j.gastro.2009.06.005

33. Poynard T, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D et al (2005) The diagnostic value of biomarkers (SteaToTest) for the prediction of liver steatosis. Comp Hepatol 4:10. https://doi.org/10.1186/1476-5926-4-10

34. Pettai A, Amato M, Cabidi D, Camma C, Di Marco V, Giordano C et al (2010) visceral adiposity index is associated with histological findings and high viral load in patients with chronic hepatitis C due to genotype 1. Hepatology 52:1543–1552. https://doi.org/10.1002/hep.23859

35. Guererro-Romero F, Simental-Mendia LE, Gonzalez-Ortiz M, Martinez-Armas E, Ramos-Zavala MG, Hernandez-Gonzalez SO et al (2010) The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. J Clin Endocrinol Metab 95:3347–3351. https://doi.org/10.1210/jc.2010-0288

36. Bedogni G, Kahn HS, Bellentani S, Tiribelli C (2010) A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterol 10:98. https://doi.org/10.1186/1471-230X-10-98

37. Kahn HS (2005) The “lipid accumulation product” performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovasc Disord 5:26. https://doi.org/10.1186/1471-2261-5-26

38. Fedchuk L, Nascimbeni F, Pais R, Charlotte F, House C, Ratziu V (2014) Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther 40:1209–1222. https://doi.org/10.1111/apt.12963

39. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease (2016) Diabetologia 59:1121–1140. https://doi.org/10.1007/s00125-016-3902-y

40. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E et al (2011) Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 54:1082–1090. https://doi.org/10.1002/hep.24452

41. Stefan N, Haring HU, Cusi K (2019) Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. Lancet Diabetes Endocrinol 7:313–324. https://doi.org/10.1016/S2213-8587(18)30154-2

42. Bril F, Ortiz-Lopez C, Lomonaco R, Orsak B, Freckleton M, Chintapalli K et al (2015) Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. Liver Int 35:2139–2146. https://doi.org/10.1111/liv.12840

43. Ryan CK, Johnson LA, Germin BL, Marcos A (2002) One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. Liver Transpl 8:1114–1122. https://doi.org/10.1053/jlts.2002.36740

44. Strauss S, Gavish E, Gottlieb P, Katsnelson L (2007) Interobserver and intraobserver variability in the sonographic assessment of fatty liver. Am J Roentgenol 189:W320–W323. https://doi.org/10.2214/AJR.07.2123

45. Fujiwara Y, Kuroda H, Abe T, Ishida K, Oguri T, Noguchi S et al (2018) The B-mode image-guided ultrasound attenuation parameter accurately detects hepatic steatosis in chronic liver disease. Ultrasound Med Biol 44:2223–2232. https://doi.org/10.1016/j.ultrasmedbio.2018.06.017

46. Tada T, Iijima H, Kobayashi N, Yoshida M, Nishimura T, Kumada T et al (2019) Usefulness of attenuation imaging with an ultrasound scanner for the evaluation of hepatic steatosis. Ultrasound Med Biol 45:2679–2687. https://doi.org/10.1016/j.ultrasmedbio.2019.05.033

47. Tamaki N, Koizumi Y, Hirooka M, Yada N, Takada H, Nakashima O et al (2018) Novel quantitative assessment system of liver steatosis using a newly developed attenuation measurement method. Hepatol Res 48:821–828. https://doi.org/10.1111/hepr.13179

48. Mikolasevic I, Orlic L, Franjic N, Hauser G, Sticma D, Milic S (2016) Transient elastography (FibroScan®) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease: where do we stand? World J Gastroenterol 22:7236–7251. https://doi.org/10.3748/wjg.v22.i32.7236

49. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V et al (2017) Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 66:1022–1030. https://doi.org/10.1016/j.jhep.2016.12.022

50. Caussy C, Alquirais MH, Nguyen P, Hernandez C, Cepin S, Fortney LE et al (2018) Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. Hepatology 67:1348–1359. https://doi.org/10.1002/hep.29639

51. Petroff D, Blank V, Newsome PN, Shalimar VCS, Thiele M et al (2021) Assessment of hepatic steatosis by controlled attenuation parameter using the M and XL probes: an individual patient data meta-analysis. Lancet Gastroenterol Hepatol 6:185–198. https://doi.org/10.1016/heap.29639
Ferraioli G, Tinelli C, Lissandrini R, Zicchetti M, Rondanelli M, Perani G et al (2014) Interobserver reproducibility of the controlled attenuation parameter (CAP) for quantifying liver steatosis. Hepatol Int 8:576–581. https://doi.org/10.1007/s12072-014-9573-1

de Lédinghen V, Vergniol J, Capdepont M, Chermak F, Hiriart JB, Cassinotto C et al (2014) Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. J Hepatol 60:1026–1031. https://doi.org/10.1016/j.jhep.2013.12.018

Sasso M, Audière S, Kemgang A, Gaouar F, Corpechot C, Chazouillères O et al (2016) Liver steatosis assessed by controlled attenuation parameter (CAP) measured with the XL probe of the FibroScan: a pilot study assessing diagnostic accuracy. Ultrasound Med Biol 42:92–103. https://doi.org/10.1016/j.ultrasmedbio.2015.08.008

Caussy C, Brissot J, Singh S, Bassirian S, Hernandez C, Bettencourt R et al (2020) Prospective, same-day, direct comparison of controlled attenuation parameter with the M vs the XL Probe in patients with nonalcoholic fatty liver disease, using magnetic resonance imaging-proton density fat fraction as the standard. Clin Gastroenterol Hepatol 18:1842-1850.e6. https://doi.org/10.1016/j.cgh.2019.11.060

Lee YH, Cho Y, Lee BW, Park CY, Lee DH, Cha BS et al (2019) Nonalcoholic fatty liver disease in diabetes. Part I: epidemiology and diagnosis. Diabetes Metab J 43:31–45. https://doi.org/10.4093/dmj.2019.0011

Chartampillas E (2018) Imaging of nonalcoholic fatty liver disease and its clinical utility. Hormones (Athens) 17:69–81. https://doi.org/10.1007/s42000-018-0012-x

PiekarSKI J, Goldberg HI, Royal SA, Axel L, Moss AA (1980) Three-dimensional proton density weighted fast spin-echo imaging: a new method for the noninvasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: clinical trials to clinical practice. J Hepatol 65:1006–1016. https://doi.org/10.1016/j.jhep.2016.06.005

Loomba R, Sirlin CB, Ang B, Bettencourt R, Jain R, Salotti J et al (2015) Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). Hepatology 61:1239–1250. https://doi.org/10.1002/hep.27647

Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R et al (2017) Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: a long-term follow-up study. Hepatol Commun 2:48–57. https://doi.org/10.1002/hep4.1124

Grgujević I, Podrug K, Mikolasevic I, Kukla M, Madir A, Tsochatzis EA (2020) Natural history of nonalcoholic fatty liver disease: implications for clinical practice and an individualized approach. Can J Gastroenterol Hepatol 2020:918368. https://doi.org/10.1155/2020/918368

Nasr P, Forsgren MF, Ignatova S, Dahlström N, Cedersund G, Leinhard OD et al (2017) Using a 3% proton density fat fraction as a cut-off value increases sensitivity of detection of hepatic steatosis, based on results from histopathology analysis. Gastroenterology 153:53-55.e7. https://doi.org/10.1016/j.gastro.2017.03.005

Bril F, Barb D, Portillo-Sanchez P, Biernacki D, Lomonaco R, Suman A et al (2017) Metabolic and histological implications of intrahepatic triglyceride content in nonalcoholic fatty liver disease. Hepatology 65:1132–1144. https://doi.org/10.1002/hep.28985

Wildman-Tobriner B, Middleton MM, Moynan CA, Rossi S, Flores O, Chang ZA et al (2018) Association between magnetic resonance imaging-proton density fat fraction and liver histology features in patients with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis. Gastroenterology 155:1428–1435. https://doi.org/10.1016/j.gastro.2018.07.018

VanWagner LB, Binell ME (2016) Extrahepatic manifestations of nonalcoholic fatty liver disease. Curr Hepat Rev 15:75–85. https://doi.org/10.1016/s11901-016-0295-9

Ginés P, Grauera I, Lammert F, Angeli P, Caballeria L, Krag A et al (2016) Screening for liver fibrosis in the general population: a call for action. Lancet Gastroenterol Hepatol 1:256–260. https://doi.org/10.1016/S2468-1253(16)30081-4

Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC et al (2007) The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 45:846–854. https://doi.org/10.1002/hep.21496

Sterling RK, Lissen E, Clumeneck N, Sola R, Correa MC, Montaner J et al (2006) Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 43:1317–1325. https://doi.org/10.1002/hep.21718

Angulo P, Bugianesi E, Björnsson ES, Chartarchaeonwittaya P, Mills PR, Barrera F et al (2013) Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 145:782-789.e4. https://doi.org/10.1053/j.gastro.2013.06.057
McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M et al (2017) Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol 112:740–751. https://doi.org/10.1038/ajg.2016.453

Boursier J, Guillaume M, Leroy V, Irelis M, Roux M, Lannes A et al (2019) New sequential combinations of non-invasive fibrosis tests provide an accurate diagnosis of advanced fibrosis in NAFLD. J Hepatol 71:389–396. https://doi.org/10.1016/j.jhep.2019.04.020

Abeysekera KWM, Fernandez GS, Hammerton G, Portal AJ, Gordon FH, Heron J et al (2020) Prevalence of steatosis and fibrosis in young adults in the UK: a population-based study. Lancet Gastroenterol Hepatol 5:295–305. https://doi.org/10.1016/S2468-1253(19)30419-4

Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J et al (2010) Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut 59:1245–1251. https://doi.org/10.1136/gut.2009.203166

Daniels SJ, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A et al (2019) ADAPT: An algorithm incorporating PROC3 accurately identifies patients with NAFLD and advanced fibrosis. Hepatology 69:1075–1086. https://doi.org/10.1002/hep.30163

Lykiardopoulos B, Hagström H, Fredriksson M, Ignatova S, Stål P, Hultcrantz R et al (2016) Development of serum marker models to increase diagnostic accuracy of advanced fibrosis in non-alcoholic fatty liver disease: the new LINX1 algorithm compared with established algorithms. PLoS One 11(12):e0167776. https://doi.org/10.1371/journal.pone.0167776

Ampuero J, Pais R, Aller R, Gallego-Durán R, Crespo J, García-Monzón C et al (2020) Development and validation of hepatitis fibrosis scoring system—a simple, noninvasive test to identify patients with nonalcoholic fatty liver disease with advanced fibrosis. Clin Gastroenterol Hepatol 18:216–225. https://doi.org/10.1016/j.cgh.2019.05.051

Ampuero J, Aller R, Gallego-Durán R, Banales J, Crespo J, Mora-Cuadrado N et al (2018) Clinical outcomes in biopsy-proven NAFLD patients from the HEPAMet Pan-American Registry. J Hepatol 68(Suppl 1):S833

Åberg F, Danford CJ, Thiele M, Talbäck M, Rasmussen DN, Jiang ZG et al (2021) A dynamic aspartate-to-alanine aminotransferase ratio provides valid predictions of incident severe liver disease. Hepatol Commun 5:1021–1035. https://doi.org/10.1002/hepc.17900

McPherson S, Stewart SF, Henderson E, Burt AD, Day CP (2010) Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 59:1265–1269. https://doi.org/10.1136/gut.2010.216077

Sun W, Cui H, Li N, Wei Y, Lai S, Yang Y et al (2016) Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: a meta-analysis study. Hepatol Res 46:862–870. https://doi.org/10.1111/hepr.12647

Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanjay AJ (2009) Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 7:1104–1112. https://doi.org/10.1016/j.cgh.2009.05.033

Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA (2008) Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 57:1441–1447. https://doi.org/10.1136/gut.2007.146019

Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez N et al (2011) Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. J Hepatol 54:160–163. https://doi.org/10.1016/j.jhep.2010.06.028

Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S et al (2008) Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 47:455–460. https://doi.org/10.1002/hep.21984

Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D et al (2004) Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 127:1704–1713. https://doi.org/10.1053/j.gastro.2004.08.052

Cales P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A et al (2005) A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology 42:1373–1381. https://doi.org/10.1002/hep.20935

Ratziu V, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L et al (2006) Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 6:6. https://doi.org/10.1186/1471-230X-6-6

Ozturk A, Grajo JR, Dhayani M, Anthony BW, Samir AE (2018) Principles of ultrasound elastography. Abdom Radiol (NY) 43:773–785. https://doi.org/10.1007/s00261-018-1475-6

Papatheodoridi M, Hiriart JB, Lupson-Platon M, Bronte F, Boursier J, Elshaarawy O, Marra F et al (2021) Refining the Baveno VI elastography criteria for the definition of compensated advanced chronic liver disease. J Hepatol 74:1109–1116. https://doi.org/10.1016/j.jhep.2020.11.050

Shen F, Zheng RD, Shi JP, Mi YQ, Chen GF, Hu X et al (2015) Impact of skin capsular distance on the performance of controlled attenuation parameter in patients with chronic liver disease. Liver Int 35:2392–2400. https://doi.org/10.1111/liv.12809

Yvuppalanchi R, Siddiqui MS, Van Natta ML, Hallinan E, Brandman D, Kowdley K et al (2018) Performance characteristics of vibration-controlled transient elastography for evaluation of non-alcoholic fatty liver disease. Hepatology 67:134–144. https://doi.org/10.1002/hep.29489

Zhou JH, Cai JJ, She ZG, Li HL (2019) Noninvasive evaluation of nonalcoholic fatty liver disease: current evidence and practice. World J Gastroenterol 25:1307–1326. https://doi.org/10.3748/wjg.v25.i11.1307

Cassinotto C, Boursier J, de Ledinghen V, Lebigot J, Lapuyade B, Cales P et al (2016) Liver stiffness in nonalcoholic fatty liver disease: a comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. Hepatology 63:1817–1827. https://doi.org/10.1002/hep.28394

Venkatesh SK, Yin M, Ehmam RL (2013) Magnetic resonance elastography of liver: technique, analysis, and clinical applications. J Magn Reson Imaging 37:544–555. https://doi.org/10.1002/jmri.23731

Singh S, Venkatesh SK, Loomba R, Wang Z, Sirlin C, Chen J et al (2016) Magnetic resonance elastography for staging liver fibrosis in non-alcoholic fatty liver disease: a diagnostic accuracy systematic review and individual participant data pooled analysis. Eur Radiol 26:1431–1440. https://doi.org/10.1007/s00330-015-3949-z

Kim D, Kim WK, Talwalkar JA, Kim HJ, Ehmam RL (2013) Advanced fibrosis in nonalcoholic fatty liver disease: noninvasive assessment with MR elastography. Radiology 268:411–419. https://doi.org/10.1148/radiol.13121193

Loomba R, Wolfson T, Ang B, Hooker J, Behling C, Peterson M et al (2014) Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a
prospective study. Hepatology 60:1920–1928. https://doi.org/10.1002/hep.27362

107. Krawczyk M, Liebe R, Lamert F (2020) Toward genetic prediction of nonalcoholic fatty liver disease trajectories: PNPLA3 and beyond. Gastroenterology 158:1865–1880. https://doi.org/10.1053/j.gastro.2020.01.053

108. Romeo S, Kozlitina J, Xing C, Pertsemilidis A, Cox D, Pennachio LA et al (2008) Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 40:1461–1465. https://doi.org/10.1038/ng.257

109. Wang Y, Kory N, BasuRay S, Cohen JC, Hobbs HH (2019) PNPLA3, CGI-58, and inhibition of hepatic triglyceride hydrolysis in mice. Hepatology 69:2427–2441. https://doi.org/10.1002/hep.30583

110. Ajmera V, Liu A, Bettencourt R, Dhar D, Richards L, Loomba R (2021) The impact of genetic risk on liver fibrosis in non-alcoholic fatty liver disease as assessed by magnetic resonance elastography. Aliment Pharmacol Ther 54:68–77. https://doi.org/10.1111/apt.16392

111. Eslam M, Valenti L, Romeo S (2018) Genetics and epigenetics of NAFLD and NASH: clinical impact. J Hepatol 68:268–279. https://doi.org/10.1016/j.jhep.2017.09.003

112. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjerg-Hansen A et al (2014) Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 46:352–356. https://doi.org/10.1038/ng.2901

113. Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JBS et al (2014) TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with nonalcoholic fatty liver disease. Nat Commun 5:4309. https://doi.org/10.1038/ncomms5309

114. Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PRV, Orho-Melander M et al (2009) The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 18:4081–4088. https://doi.org/10.1093/hmg/ddp357

115. Tan HL, Zain SM, Mohamed R, Rampal S, Chin KH, Basu RC et al (2014) Association of glucokinase regulatory gene polymorphisms with risk and severity of nonalcoholic fatty liver disease: an interaction study with adiponutrin gene. J Gastroenterol 49:1056–1064. https://doi.org/10.1007/s00535-013-0850-x

116. Yu C, Xu C, Xu L, Yu J, Miao M, Li Y (2012) Serum proteomic analysis revealed diagnostic value of hemoglobin for nonalcoholic fatty liver disease. J Hepatol 56:241–247. https://doi.org/10.1016/j.jhep.2011.05.027

117. Kalhan SC, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW et al (2011) Plasma metabolic profile in nonalcoholic fatty liver disease. Metabolism 60:404–413. https://doi.org/10.1016/j.metabol.2010.03.006

118. Oresic M, Hyotylainen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J et al (2013) Prediction of non-alcoholic fatty liver disease and liver fat content by serum molecular lipids. Diabetologia 56:2266–2274. https://doi.org/10.1007/s00125-013-2981-2

119. Mayo R, Crespo J, Martinez-Arranz I, Banales JM, Arias M, Minchola I et al (2018) Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. Hepatol Commun 2:807–820. https://doi.org/10.1002/hepc.4.1188

120. Pos O, Biro O, Szemes T, Nagy B (2018) Circulating cell-free nucleic acids: Characteristics and applications. Eur J Hum Genet 26:937–945. https://doi.org/10.1038/s41431-018-0132-4

121. Turchinovich A, Baranova A, Drapkin O, Tonevitsky A (2018) Cell-free circulating nucleic acids as early biomarkers for NAFLD and NASH-derived-associated disorders. Front Physiol 9:1256. https://doi.org/10.3389/fphys.2018.01256

122. Tan Y, Ge G, Pan T, Wen D, Gan J (2014) A pilot study of serum microRNAs panel as potential biomarkers for diagnosis of non-alcoholic fatty liver disease. PLoS ONE 9:e105192. https://doi.org/10.1371/journal.pone.0105192

123. Johnson K, Leary PJ, Govaere O, Barter MJ, Charlton SH, Cockell SJ et al (2021) Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: Diagnostic and mechanistic relevance. JHEP Rep 4:100409. https://doi.org/10.1016/j.jheprep.2021.100409

124. Aron-Wisnewsky J, Gaborit B, Doutour A, Clement K (2013) Gut microbiota and non-alcoholic fatty liver disease: new insights. Clin Microbiol Infect 19:338–348. https://doi.org/10.1111/1469-0002.12485

125. Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG (2017) Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. Hepatobiliary Pancreat Dis Int 16:375–381. https://doi.org/10.1016/j.sjgp.2017.06.009

126. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F et al (2016) The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology 63:764–775. https://doi.org/10.1002/hep.28356

127. Loomba R, Seguirian V, Li W, Long T, Kligord N, Bhatt A et al (2017) Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab 25:1054–1062. https://doi.org/10.1016/j.cmet.2017.04.001

128. Lang S, Farowski F, Martin A, Wispelhoff H, Vehreschild MIGT, Krawczyk M et al (2020) Prediction of advanced fibrosis in non-alcoholic fatty liver disease using gut microbiota-based approaches compared with simple non-invasive tools. Sci Rep 10:9385. https://doi.org/10.1038/s41598-020-66241-0

129. Llop E, Iruzubieta P, Perelló C, Cabezas J, Escudero-Garcia D, Gonzáles M et al (2018) Transient elastography as a method of screening of chronic hepatic disease in apparently healthy population. Results from ETHON cohort J Hepatol 68(Suppl 1):S634

130. Zhang E, Wartelle-Bladou C, Lepanto L, Lachaine J, Cloutier G, Tang A (2015) Cost-utility analysis of nonalcoholic steatohepatitis screening. Eur Radiol 25:3282–3294. https://doi.org/10.1007/s00330-015-3731-2

131. Phisalprapa P, Supakankunti S, Charatcharoenwitthaya P, Apisantharanak P, Charoesanak A, Washirasakski C et al (2017) Cost-effectiveness analysis of ultrasonography screening for nonalcoholic fatty liver disease in metabolic syndrome patients. Medicine (Baltimore) 96:e6585. https://doi.org/10.1097/MD.0000000000006585

132. Tanajewski L, Harris R, Harman DJ, Aithal GP, Card TR, Gkountouras G et al (2017) Economic evaluation of a community-based diagnostic stratification tool for adults with nonalcoholic fatty liver disease: a Markov model informed by a feasibility study. BMJ Open 7:e015659. https://doi.org/10.1136/bmjopen-2016-015659

133. Caballeria L, Pera G, Arteaga I, Rodriguez L, Aluma A, Morillas RM et al (2018) High prevalence of liver fibrosis among European adults with unknown liver disease: a population-based study. Clin Gastroenterol Hepatol 16(1138–1145):e1135. https://doi.org/10.1016/j.cgh.2017.12.048

134. Koehler EM, Plomp EM, Schouten JN, Hansen BE, Darwish Murad S, Taimr P et al (2016) Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: the Rotterdam study. Hepatology 63:138–147. https://doi.org/10.1002/hep.27981

135. Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S et al (2011) Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut 60:977–984. https://doi.org/10.1136/gut.2010.221382
136. Sporea I, Mare R, Popescu A, Nistorescu S, Baldea V, Sirli R et al (2020) Screening for liver fibrosis and steatosis in a large cohort of patients with type 2 diabetes using vibration controlled transient elastography and controlled attenuation parameter in a single-center real-life experience. J Clin Med 9:1032. https://doi.org/10.3390/jcm9041032

137. Lai LL, Wan Yusoff WNI, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK (2019) Screening for non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus using transient elastography. J Gastroenterol Hepatol 34:1396–1403. https://doi.org/10.1111/jgh.14577

138. Roulot D, Roudot-Thoraval F, NKontchou G, Kouacou N, Costes JL, Elourimi G et al (2017) Concomitant screening for liver fibrosis and steatosis in French type 2 diabetic patients using Fibrocan. Liver Int 37:1897–1906. https://doi.org/10.1111/liv.13481

139. Di Cesare M, Soric M, Bovet P, Miranda JJ, Bhutta Z, Stevens GA et al (2019) The epidemiological burden of obesity in childhood: a worldwide epidemic requiring urgent action. BMC Med 17:212. https://doi.org/10.1186/s12876-019-1449-8

140. Type 2 diabetes: the urgent need to protect young people (2018) Lancet 392:2325. https://doi.org/10.1016/S0140-6736(18)33015-0

141. Lascar N, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S (2018) Type 2 diabetes in adolescents and young adults. Lancet Diabetes Endocrinol 6:69–80. https://doi.org/10.1016/S2213-8587(17)30186-9

142. Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW (1990) The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 11:74–80. https://doi.org/10.1002/hep.1840110114

143. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, Castellanos M, Aller-de la Fuente R, Metwally M et al (2018) Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. Gastroenterology 155(443–57):e17. https://doi.org/10.1053/j.gastro.2018.04.034

144. Nasr P, Iredahl F, Dahlström N, Rådholm K, Henriksen P, Cedersund G et al (2021) Evaluating the prevalence and severity of NAFLD in primary care: the EPSONID study protocol. BMC Gastroenterol 21:180. https://doi.org/10.1186/s12876-021-01763-z

145. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G (2017) Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. Hepatology 66:1486–1501. https://doi.org/10.1002/hep.2930

146. Ansee QM, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T et al (2019) Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR trials. Hepatology 70:1521–1530. https://doi.org/10.1002/hep.30842

147. Guillaume M, Moal V, Delabaudiere C, Zuberbuhler F, Robic MA, Lannes A et al (2019) Direct comparison of the specialised blood fibrosis tests FibroMeter(V2G) and Enhanced Liver Fibrosis score in patients with non-alcoholic fatty liver disease from tertiary care centres. Aliment Pharmacol Ther 50:1214–1222. https://doi.org/10.1111/apt.15529

148. Herrmann E, de Ledinghen V, Cassinotto C, Chu WC, Leung YY, Ferrarioli G et al (2018) Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: an individual patient data-based meta-analysis. Hepatology 67:260–272. https://doi.org/10.1002/hep.29179

149. Jiang W, Huang S, Teng H, Wang P, Wu M, Zhou X et al (2018) Diagnostic accuracy of point shear wave elastography and transient elastography for staging hepatic fibrosis in patients with non-alcoholic fatty liver disease: a meta-analysis. BMJ Open 8:e021787. https://doi.org/10.1136/bmjopen-2018-021787

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.