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Non-invasive methods for imaging hepatic steatosis and their clinical importance in NAFLD

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Abstract

Hepatic steatosis is a key histological feature of nonalcoholic fatty liver disease (NAFLD). The non-invasive quantification of liver fat is now possible due to advances in imaging modalities. Emerging data suggest that high levels of liver fat and its temporal change, as measured by quantitative non-invasive methods, might be associated with NAFLD progression. Ultrasound-based modalities have moderate diagnostic accuracy for liver fat content and are suitable for screening. However, of the non-invasive imaging modalities, MRI-derived proton density fat fraction (MRI-PDFF) has the highest diagnostic accuracy and is used for trial enrolment and to evaluate therapeutic effects in early-phase clinical trials in nonalcoholic steatohepatitis (NASH). In patients with NAFLD without advanced fibrosis, high levels of liver fat are associated with rapid disease progression. Furthermore, changes on MRI-PDFF (≥30% decline relative to baseline) are associated with NAFLD activity score improvement and fibrosis regression. However, an inverse association exists between liver fat and complications of cirrhosis. Liver fat decreases as liver fibrosis progresses towards cirrhosis, and the clinical importance of quantitative measurements of liver fat differs by NAFLD status. As such, patients with NAFLD should be stratified by fibrosis severity to investigate the utility of quantitative measurements of liver fat for assessing NAFLD progression and prognosis

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

R.L. serves as a consultant or advisory board member for Anylam/Regeneron, Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Cirius, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse bio, GNI, GRI Bio, Inipharm, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Promethera, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirius, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Grail, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, pH Pharma, Prometheus, and Siemens. He is also co-founder of Liponexus, Inc. The other authors declare no competing interests.

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Nonalcoholic fatty liver disease (NAFLD) affects over 25% of the global population and is a common cause of chronic liver disease[1,2]. The number of patients with NAFLD has increased in parallel with the global increase in the prevalence of obesity and the metabolic syndrome[3]. Hepatic steatosis, which is characterized by the abnormal accumulation of lipids in the cytoplasm of hepatocytes, is one of the main features of NAFLD and is detected by both histology and non-invasive imaging[4]. NAFLD is associated with increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD), and has also been linked to hepatocellular carcinoma (HCC)[5–7]. Other chronic liver diseases also frequently coexist with hepatic steatosis, and the progression of chronic liver disease can be modified by liver lipids[8,9]. Therefore, accurate evaluation and quantification of liver fat is becoming clinically important.

Liver biopsy is a reference standard for the evaluation of histological features of chronic liver disease, including steatosis grade[10]. Steatosis grade by a liver biopsy is expressed as the percentage of hepatocytes with lipid deposits and is stratified into four grades (grade 0, <5%; grade 1, 5–33%; grade 2, 34–66%; grade 3, >66%)[11]. Of note, liver biopsy has several limitations, including invasiveness, sampling error, and only moderate intraobserver and interobserver reproducibility[12]. These limitations constrain the use of liver biopsy as a repeat measurement to investigate histological changes. To fulfil this clinical need, several non-invasive, quantitative and objective methods for evaluation of histological features have been developed[13,14]. Hepatic steatosis can be detected by conventional B-mode ultrasonography; however, conventional B-mode ultrasonography is limited due to its subjective and examiner-dependent interpretation, and its inability to quantify liver fat[13]. Therefore, objective, examiner-independent and quantitative methods have emerged to more accurately quantify liver fat using both ultrasonography and MRI-based methods, such as MRI-derived proton density fat fraction (MRI-PDFF). Furthermore, emerging data suggest that liver fat and its temporal change, as measured by non-invasive quantitative modalities, might be associated with NAFLD progression[15–17].

In this Review, we discuss currently available and emerging imaging modalities for the quantification of liver fat. Furthermore, we highlight the clinical utility of liver lipid quantification and its association with disease progression in NAFLD. Finally, we consider the role of liver lipid quantification in the assessment of treatment response in nonalcoholic steatohepatitis (NASH).

Imaging assessment of liver fat

This section describes the various non-invasive imaging modalities that are available to assess hepatic steatosis. The clinical utility and limitations of each imaging modality for the assessment of liver fat are summarized in TABLE 1.

Conventional B-mode ultrasonography

Conventional B-mode ultrasonography is widely used in screening and health check-ups owing to its ease of use and low cost. When using this modality to image the liver, parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, bright vessel walls and gallbladder wall definition are findings associated with liver fat; the presence of one or

more of these ultrasonic features is defined as hepatic steatosis[18]. However, the diagnosis of hepatic steatosis by conventional B-mode ultrasonography has some limitations. Although conventional B-mode ultrasonography has high diagnostic accuracy for the presence of moderate to severe hepatic steatosis (≥30% liver fat), the sensitivity for mild steatosis (<30% liver fat) is lower[18–20]. Furthermore, the diagnostic accuracy of conventional B-mode ultrasonography is lower in patients with obesity than in those without[21,22]. Of note, the definition of NAFLD includes the presence of ≥5% hepatic steatosis on histology, and identification of patients with NAFLD who have mild steatosis by conventional B-mode ultrasonography is usually limited. Although some studies attempted to evaluate liver fat by semiquantitative scoring[23,24], B-mode ultrasonography is not quantitative. Another limitation is that B-mode ultrasonography is examiner-dependent, lacks sensitivity and is subjective; therefore, interobserver and intraobserver reproducibility are not high[25].

Ultrasound-based quantitative liver lipid measurement

Several technologies provide improved assessment of liver fat compared with conventional B-mode ultrasonography by implementing quantitative approaches. Controlled attenuation parameter (CAP), ultrasound-guided attenuation parameter (UGAP), attenuation coefficient (ATT) and attenuation imaging (ATI) quantify liver fat by measuring the attenuation of radiofrequency. The amount of radiofrequency decay varies depending on the tissue, and echo attenuation is larger in liver with any grade of hepatic steatosis than in normal liver. Methods for quantifying liver fat by quantifying the amount of attenuation have been developed and used in clinical practice[26]. In addition, backscatter coefficient (BSC) is a quantitative value that reflects ultrasonic pulses that are scattered back to an echo probe after passing through tissue. The number of scattered back ultrasonic pulses increases with increasing levels of liver fat. Therefore, BSC might also have potential to detect and quantify hepatic steatosis. Finally, quantitative ultrasonography (QUS) for fat fraction estimation that quantifies liver fat using two backscatter parameters has been developed. We discuss the attributes and limitations of each of these modalities below.

CAP.—CAP was the first approved method for the quantification of liver fat based on attenuation evaluation. CAP is widely used to assess hepatic steatosis and its diagnostic accuracy has been validated extensively. For example, a meta-analysis that compared histologically graded steatosis with CAP and included 19 studies (2,735 patients with chronic liver disease) found areas under the receiver operating characteristics (AUROC) of 0.82 with the 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%[27]. However, the CAP value is affected by the presence of NAFLD, diabetes mellitus and obesity[27]. In patients with NAFLD, the optimal CAP thresholds for detecting MRI-PDFF of ≥5% and of ≥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 aetiologies of liver disease[28].

Limited data directly compare the diagnostic accuracy of B-mode ultrasonography and CAP. However, a meta-analysis published in 2021 investigated 2,346 patients with chronic liver disease and demonstrated that the accuracy of CAP is good in patients with NAFLD in

detecting the presence of any histological steatosis grades 1–3 versus grade 0 (AUROC 0.819)[29]. Furthermore, CAP has good diagnostic accuracy for MRI-PDFF ≥5% and ≥10%[28]. As the diagnostic accuracy for mild hepatic steatosis (liver fat content 5–30%) is limited with conventional B-mode ultrasonography, CAP could be especially suitable for detecting mild steatosis. Furthermore, CAP has high interobserver reproducibility of 0.82 (REF.[30]), and this feature is an advantage of CAP compared with conventional B-mode ultrasonography.

One limitation of CAP is that it has a high rate of measurement failure (0–24%)[13]. The measurement failure rate was 7.7% in a study examining 5,323 patients with chronic liver disease. Female sex, BMI ≥30 kg/m^2 and the presence of the metabolic syndrome were factors associated with measurement failure[31]. To reduce the rates of measurement failure and invalid results, an obesity-specific probe (known as the XL probe) was developed[32]. The interquartile range (IQR) of CAP is associated with its diagnostic accuracy; using an IQR of 40 dB/cm as the criterion for M probe (the original probe) validity can reduce the rate of invalid results[33]. The diagnostic accuracies of the M probe and XL probe are equivalent[34,35]; however, some studies have demonstrated that in patients with NAFLD, the threshold for detecting liver fat is higher with the XL probe than with the M probe[35,36]. Therefore, the optimal thresholds with the M probe and XL probe should be verified, especially in patients with NAFLD.

UGAP, ATT and ATI.—One disadvantage of CAP is that it is blind to the exact location of the region of interest. This factor contributes to high rates of measurement failure (0–24%). To resolve this issue, methods that can measure liver fat content on a conventional B-mode ultrasound image in real-time with exact localization of the region of interest have been developed (UGAP, ATT and ATI). These modalities measure liver fat using similar principles (attenuation measurement) and have been developed independently of each other. These modalities have been approved for use in clinical practice.

In a study investigating the diagnostic accuracy of UGAP in 163 patients with chronic liver disease, the median UGAP values for histological steatosis grade 0 (<5%), 1 (5–33%), 2 (34–66%) and 3 (>66%) were 0.49, 0.56, 0.66 and 0.72 dB/cm/MHz, respectively, and the UGAP values statistically significantly increased as the steatosis grade increased[37]. In this study, the diagnostic accuracy of UGAP for detecting histological steatosis grade was higher than that of CAP. Furthermore, the measurement failure rate for CAP was 5.2% of patients, whereas for UGAP no patient had measurement failure. The correlation coefficient for the comparison between UGAP and MRI-PDFF was 0.746, which was statistically significant, and a significant correlation was also observed in patients with obesity[38].

The diagnostic accuracy of ATT was investigated in 351 patients with biopsy-proven chronic liver disease. The median ATT values for histological steatosis grades 0, 1, 2 and 3 were 0.55, 0.63, 0.69 and 0.85 dB/cm/MHz, respectively, and the ATT values increased with increasing steatosis grade[39]. The diagnostic accuracies of ATT and CAP for histological steatosis grade were equivalent; however, ATT had the advantage of no measurement failure in any patient (5.3% measurement failure with CAP)[40]. In addition, the measurement

accuracy among the different examiners also showed high agreement with an intraclass correlation coefficient of 0.907 (REF.[41]).

A study compared ATI measurement with histological steatosis grade in 148 patients with chronic liver disease and found that the median ATI values for steatosis grades 0, 1, 2 and 3 were 0.60, 0.64, 0.78 and 0.86 dB/cm/MHz, respectively. In addition, ATI values increased with increasing steatosis grade[42]. A statistically significant correlation between ATI measurement and histological steatosis grade was also confirmed in another cohort of 108 patients with chronic liver disease, with no measurement failure[43]. A significant correlation (r = 0.70–0.81) was also found between MRI-PDFF and ATI measurement in different cohorts of patients with chronic liver disease[44,45.]

An advantage of UGAP, ATT and ATI is that these modalities can measure liver fat on conventional ultrasound B-mode imaging in real time, with exact localization of the region of interest. The diagnostic accuracies of these modalities are equivalent to that of CAP, with lower rates of measurement failure. However, the studies investigating the accuracy of these modalities included fairly small numbers of patients compared with those investigating the accuracy of CAP. As such, future studies including greater numbers of patients are necessary to confirm the utility of these modalities in large populations. In addition, optimal disease-specific cut-off points will need to be verified; for example, for patients with diabetes mellitus or obesity.

QUS for PDFF estimation.—QUS is a technique under investigation that estimates PDFF by measuring acoustic parameters such as BSC. A study demonstrated that quantitative BSC values correlate with MRI-PDFF and that patients with NAFLD (defined as MRI-PDFF ≥5%) could be identified by BSC, with an AUROC of 0.98 (REF.[46]). Furthermore, BSC had a higher accuracy in quantifying liver fat than conventional ultrasonography, using liver biopsy as the reference standard[47]. Of note, in patients with NAFLD, BSC measurement showed a high interobserver and interplatform reproducibility[48,49.] A study that investigated seven ultrasound parameters in 102 patients with NAFLD demonstrated that two quantitative ultrasound scatter parameters are significantly associated with MRI-PDFF[50]. Furthermore, this study found that QUS for PDFF estimation based on the two quantitative parameters is correlated with MRI-PDFF (r = 0.76). MRI-PDFF is the most accurate non-invasive method for the assessment of liver fat (detailed in the next section), and QUS for PDFF estimation that approximates MRI-PDFF will become available as part of routine ultrasonography. Further studies are needed to validate the utility of QUS for detecting hepatic steatosis and for assessing longitudinal changes in hepatic steatosis in the setting of clinical trials for NASH therapeutics.

MRI-PDFF

MRI-PDFF is a quantitative imaging biomarker that enables accurate and precise quantitative assessment of liver fat with high intraobserver and interobserver reproducibility[13]. 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. Although MRI-PDFF and histological

steatosis grade are not equivalent, many studies have demonstrated statistically significant correlations between liver fat quantified by MRI-PDFF and steatosis grade as assessed by liver histology[51,52]. In a study that compared the diagnostic accuracies of MRI-PDFF and CAP for liver fat assessment in patients with NAFLD, MRI-PDFF had higher diagnostic accuracy than CAP when liver biopsy was used as the reference standard[53,54]. In a meta-analysis including 1,100 patients with chronic liver disease, the AUROC values of MRI-PDFF for classifying histological steatosis grades ≥1, ≥2 and ≥3 were 0.91–0.98 (REF.[55]).

One limitation of CAP is that the diagnostic accuracy in patients with NAFLD is lower than that of MRI-PDFF[27,28]. When the diagnostic accuracy of MRI-PDFF in patients with NAFLD was investigated, the summary AUROC values of MRI-PDFF for classifying histological steatosis grades ≥1, ≥2 and ≥3 were 0.98, 0.91, and 0.90, respectively, and the diagnostic accuracy was not lower in patients with NAFLD[56]. Of the non-invasive imaging modalities, MRI-PDFF currently has the highest diagnostic accuracy for hepatic steatosis in patients with NAFLD and is considered the most accurate method for quantitative measurements of liver fat.

Liver fat content and NAFLD progression

The clinical effect of hepatic steatosis on NAFLD progression is discussed in this section, including disease mechanisms and clinical outcomes.

Mechanisms of hepatic steatosis

The presence of hepatic steatosis is one of the defining histological features of NAFLD and steatosis of ≥5% is a critical component of the diagnosis of NAFLD. In the liver, the accumulation of lipids in hepatocytes is associated with progression of inflammation, fibrosis and carcinogenesis (FIG. 1). The main sources of free fatty acids (FFAs) in the liver are influxes from adipose tissue, dietary FFAs and de novo lipogenesis[57]. In hepatocytes, FFAs can enter the mitochondria and undergo β-oxidation to produce energy. FFAs are also esterified to triglycerides, which can be stored as lipid droplets or exported to blood as VLDL. In NAFLD, the metabolism of lipids is disrupted in the liver: the influx of FFAs increases, β-oxidation and secretion of VLDL decrease, and consequently the accumulation of intrahepatic triglycerides increases.

Although triglyceride is the major lipid class contained in lipid droplets, triglycerides themselves are not lipotoxic[58]. When increased levels of intrahepatic FFAs exceed the capacity of triglyceride synthesis and storage, the abundance of lipotoxic species such as palmitic acid, ceramides and lysophosphatidylcholine increases[59]. These toxic lipids can cause toxicity by inducing endoplasmic reticulum stress, causing modification of mitochondrial function and inducing oxidative stress, which all result in cell injury and death[60]. Damaged hepatocytes release signals to promote the regeneration of hepatocytes and these signals induce activation and recruitment of hepatic stellate cells, progenitor cells and immune cells, which can cause inflammation, fibrosis and carcinogenesis[61]. Genetic backgrounds affect the levels of intrahepatic FFAs and the accumulation of triglycerides in hepatocytes by increasing de novo lipogenesis, and decreasing lipolysis and VLDL secretion

(detailed in the next section). In this way, excessive accumulation of lipids in the liver causes disease progression.

Although the accumulation of lipids in the liver causes NAFLD progression to NASH, fibrosis or cirrhosis, liver fat decreases paradoxically as liver fibrosis increases to advanced fibrosis or cirrhosis, a phenomenon known as 'burned-out' NASH[62]. The mechanisms underlying this phenomenon are not fully elucidated. However, adiponectin is known to be one of the causes of burned-out NASH. Adiponectin acts directly on hepatocytes and has protective effects against NAFLD progression by increasing β-oxidation, decreasing fatty acid synthesis and improving insulin sensitivity[63,64]. In patients with NAFLD and advanced fibrosis, serum levels of adiponectin increase and elevated adiponectin is associated with decreases in liver fat. This adiponectin increase might contribute to the development of burned-out NASH[65].

Genetic factors

Several genetic risk factors are associated with NAFLD incidence and progression. The risk variant with the greatest effect on the development and progression of NAFLD and cirrhosis is the rs738409 C>G single nucleotide polymorphism of *PNPLA3*, which encodes the I148M protein variant of PNPLA3 (REF.[66]). *PNPLA3* rs738409 C>G is significantly associated with accumulation of intrahepatic lipids. Although *PNPLA3* itself encodes an enzyme with hydrolase activity towards triglycerides, cumulative evidence suggests the primary effects of the I148M protein variant on liver might be attributed to its *trans*-repression of lipase activity[67,68]. In patients with NAFLD, liver fat increases with the number of risk alleles (the G allele). For example, in a meta-analysis that included over 10,000 patients with NAFLD, the pooled odds ratios for the presence of NAFLD for rs738409 CG and rs738409 GG compared with rs738409 CC were 1.46 (95% CI 1.16–1.85) and 2.76 (95% CI 2.30–3.13), respectively. In a 2021 study of 264 patients phenotyped by MRI-PDFF, each copy of the PNPLA3 risk allele was associated with a 2.56% (95% CI 1.39–3.72, $P < 0.01$) increase in hepatic steatosis[69]. Furthermore, the pooled odds ratios for the presence of NASH for rs738409 CG and rs738409 GG compared with rs738409 CC were 1.75 (95% CI 1.24–2.46) and 4.44 (95% CI 2.96–6.76), respectively[70]. *PNPLA3* is also associated with the progression of chronic liver disease including fibrosis progression, HCC development, liver-related events and all-cause mortality, not only in NAFLD[71] but also in viral hepatitis and alcohol-associated liver disease[72,73].

The gene encoding transmembrane 6 superfamily member 2 (*TM6SF2*) is also associated with NAFLD progression. *TM6SF2* has a role in VLDL secretion, and the *TM6SF2* E167K variant results in a loss of this function, leading to the accumulation of lipids in the liver[74]. In patients with NAFLD, *TM6SF2* E167K is associated with significant fibrosis (fibrosis stages 2–4) with an odds ratio of 1.88 (95% CI 1.41–2.5), independent of *PNPLA3* genotype[75].

Glucokinase regulatory protein (GCKR) is a fructose-6-phosphate-dependent inhibitor of glucokinase and regulates de novo lipogenesis[76]. The *GCKR*^{P446L} 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)[77].

In addition to genetic risk variants in *PNPLA3*, *TM6SF2* and *GCKR*, which have well-validated associations with clinical NAFLD progression, many other risk variants (for example, *MBOAT7*, *HSD17B13* and *MERTK*) also have reported associations with NAFLD[78]. Intrahepatic lipid accumulation occurs due to a combination of genetic and environmental factors, and the presence of high levels of liver fat is notably associated with disease progression in NAFLD. Due to their influence on accumulation of lipids in the liver, these genetic risk variants further support the hypothesis that high levels of liver fat are associated with progression of chronic liver disease.

Clinical outcomes of patients with NAFLD

Patients with NAFLD are at greater risk of HCC, liver-related events and all-cause mortality compared with the general population[5,6]. A meta-analysis demonstrated increased mortality due to liver-associated disease and CVD in patients with NAFLD compared with the general population, with an odds ratio of 1.57 (REF.[79]). Patients with NAFLD also have a high risk of extrahepatic complications such as CVD, T2DM and chronic kidney disease compared with the general population[80,81]. In a meta-analysis evaluating the association between NAFLD and CVD, the odds ratio for the development of CVD in patients with NAFLD was 1.64 and increased to 2.58 in patients with ‘severe’ NAFLD. In this study, severe NAFLD was defined by the presence of steatosis plus either elevated γ-glutamyl transferase levels or high NAFLD Fibrosis Score or high hepatic fluorodeoxyglucose uptake on PET or the presence of liver fibrosis on liver histology[82]. Therefore, the accumulation of liver fat (high liver fat) is associated with an increased risk of liver disease progression including HCC, liver-related events and all-cause mortality, as well as CVD, T2DM and chronic kidney disease compared with the general population.

Clinical use of liver fat quantification

MRI-PDFF as a tool to monitor NAFLD

Several studies have demonstrated the notable association between changes in MRI-PDFF and improvements in serum levels of aspartate aminotransferase and alanine aminotransferase (biomarkers of liver damage) as well as serum biomarkers of fibrosis or body weight[83,84]. In a study investigating the effect of the bile acid sequestrant colesevelam in patients with biopsy-proven NAFLD, a 4.9% decline in MRI-PDFF was observed at the end of the study in the treatment group, but histological steatosis grade showed no significant change[85]. In a sub-analysis of the same study, MRI-PDFF decreased with improvement in clinical parameters without change in histological steatosis grade[84]. These studies indicate that changes in MRI-PDFF might have greater sensitivity in detecting changes in disease activity than changes in histopathological steatosis.

The association between changes in MRI-PDFF and changes in histological features of NAFLD has demonstrated the utility of repeated measurements of MRI-PDFF to predict changes in histological features (TABLE 2). In patients with NAFLD, changes in

MRI-PDFF have a statistically significant positive correlation with changes in steatosis grade[86–88]. In a study examining 113 patients with NASH with paired biopsy and MRI-PDFF measurements taken at baseline and 72 weeks after treatment with obeticholic acid or placebo, the mean absolute changes in MRI-PDFF in patients with improvement, no change and worsening of steatosis were −7.4%, 0.3% and 7.7%, respectively. The optimal thresholds for steatosis improvement and worsening at 90% specificity were absolute changes of −5.1% and 5.6%, respectively[86]. In another study in 169 children with NASH who underwent repeated and paired biopsy and MRI-PDFF measurements, the mean absolute changes in MRI-PDFF in patients with improvement, no change and worsening of steatosis were −7.8%, 1.2% and 4.9%, respectively[87]. In a phase II trial of selonsertib (an inhibitor of ASK1) in patients with NASH, a decline in MRI-PDFF of −20.2% relative to baseline was observed in patients with improvement in histological steatosis grade and −0.8% in patients without improvement in steatosis grade[88]. Taken together, these findings indicate that repeat measurements of MRI-PDFF can evaluate for histological changes in steatosis grade.

In phase IIB clinical trials of new drugs for NASH, histological response (classified as NAFLD activity score (NAS; a summary score of 0–8 that incorporates steatosis grades 0–3, lobular inflammation grade 0–3 and hepatocellular ballooning grade 0–2) ≥2 points improvement with no worsening of fibrosis) has been used as an end point. Therefore, developing a non-invasive surrogate marker of histological response is an unmet need. In a sub-analysis of the MOZART trial of ezetimibe (a cholesterol-lowering drug) in patients with NASH, a decline in MRI-PDFF of −29.3% relative to baseline was observed in patients with a histological response, whereas an increase in MRI-PDFF of 2.0% relative to baseline was observed in patients without a histological response[89]. Furthermore, in another trial in patients with NASH who were treated with selonsertib (a drug targeting apoptosis signal-regulating kinase 1 inhibitor) and/or simtuzumab (a monoclonal antibody targeting LOXL2), a decline in MRI-PDFF of 25% relative to baseline was the optimal threshold for predicting a histological response, with a sensitivity of 50% and a specificity of 81%[90]. In a sub-analysis of the FLINT study of obeticholic acid (a farnesoid X receptor ligand) in patients with NASH, the optimal threshold for a histological response was a 30% decline in MRI-PDFF relative to baseline. In this analysis, the histological response rate in patients with a ≥30% decline in MRI-PDFF relative to baseline was 50%, whereas it was 19% in those without a ≥30% decline relative to baseline. The odds ratio for a histological response in patients with a ≥30% decline in MRI-PDFF relative to baseline was 4.86 (REF.[91]). Another secondary analysis of a clinical trial for resmetirom (a liver-directed, selective thyroid hormone receptor-β agonist) in patients with NASH also confirmed the positive correlation between histological response and a ≥30% decline in MRI-PDFF relative to baseline[92]. Of note, in a study investigating the effect of pioglitazone (a thiazolidinedione) in patients with NASH, the change in steatosis assessed by magnetic resonance spectroscopy was not associated with a histological response in the pioglitazone treatment group or the placebo group. However, a statistically significant association between steatosis and histological response was observed when the two groups were combined[93]. A meta-analysis published in 2020 that included seven studies and 346 patients with NAFLD who underwent repeated paired liver biopsies and MRI-PDFF assessment demonstrated that 51% of patients

with a ≥30% decline in MRI-PDFF relative to baseline achieved a histological response, with an odds ratio of 6.98 (versus 14% in patients without a ≥30% relative decline in MRI-PDFF)[16]. Therefore, histological response is strongly associated with a change in MRI-PDFF and a ≥30% decline in MRI-PDFF relative to baseline is an important end point in clinical trials in NASH.

Fibrosis regression (reduction of one or more stages by paired biopsy) is also an important end point in clinical trials in NASH. Histological response is associated with fibrosis regression[94,95]. Furthermore, a 2021 study in 100 patients with NAFLD who received a paired biopsy with contemporaneous MRI-PDFF assessment demonstrated that a ≥30% decline in MRI-PDFF relative to baseline was an independent predictor of fibrosis regression (reduction of one or more stages), with an adjusted odds ratio of 6.46 (95% CI 1.1–37.0)[17]. Based on these results, MRI-PDFF is now the preferred method for assessing patients for inclusion in phase I–IIB trials in NASH and also a biomarker of treatment response in early-phase trials in NASH for therapies that have an anti-steatotic mechanism of action.

Quantitative measurements and prognosis

The association between quantified measurements of liver fat and liver-related outcomes and mortality is summarized in TABLE 3. In patients with NAFLD, liver fibrosis is the most important histological factor associated with prognosis[96–99]. In these studies, histological steatosis grade was not associated with prognosis. As mentioned above, a phenomenon exists in NAFLD known as burned-out NASH, in which steatosis decreases with the progression of fibrosis[62]. In cross-sectional studies of patients with NAFLD, histological steatosis grade decreased as fibrosis progressed[51,100]. In a cross-sectional study comparing the characteristics of patients with NASH with and without HCC, patients with HCC had a higher proportion of advanced fibrosis and a lower proportion of moderate–severe steatosis than those without HCC[101]. Although the accumulation of lipids in the liver causes NAFLD progression, liver fat decreases with development of cirrhosis and a low proportion of patients with HCC have moderate–severe steatosis. The clinical importance of liver fat varies depending upon NAFLD status and fibrosis stage. Therefore, in order to investigate the clinical importance of liver fat on prognosis, patients should be stratified by NAFLD status and fibrosis stage. Of note, in the studies reporting no significant association between steatosis grade and prognosis, all fibrosis stage patients were combined and analysed.

In a study including 458 patients with NAFLD with advanced fibrosis (F3 and F4), overall mortality was statistically significantly higher in patients with histological steatosis <33% than in those with steatosis ≥33%[102]. In patients with advanced fibrosis, those with histological steatosis of <33% had about a two times higher odds of mortality, liver-related events and HCC development compared with those with steatosis of ≥33%. On the other hand, another study in patients with NAFLD with no baseline fibrosis demonstrated that high steatosis grade (that is, MRI-PDFF ≥15%) was associated with rapid fibrosis progression with an odds ratio of 6.67 for fibrosis progression[15]. In brief, in patients with NAFLD, high steatosis grade might be associated with rapid disease progression in patients with no or early stages of fibrosis, but steatosis grade gradually decreases with fibrosis

progression. In patients with advanced fibrosis or cirrhosis, those with a low steatosis grade might have an increased risk of progression of liver-related events and mortality.

Several studies have examined the association between CAP value and liver-related events and prognosis. In cross-sectional studies investigating the association between CAP and HCC in patients with NAFLD or other chronic liver diseases, decreased CAP values were associated with the presence of HCC[103,104]. Among patients with chronic liver disease who primarily had cirrhosis (that is, 78.5% of the cohort had cirrhosis), an increased CAP value was an independent 'protective' factor for the presence of notable portal hypertension[105]. Moreover, in patients with chronic liver disease who have advanced fibrosis (liver stiffness measurement (LSM) by vibration-controlled transient elastography ≥10 kPa), CAP ≥220 dB/m was associated with a reduced risk of hepatic decompensation with a hazard ratio of 0.043 compared with those with CAP <220 dB/m (REF.[106]). In another study in patients with chronic liver disease and advanced fibrosis (LSM ≥10 kPa), CAP did not predict the development of liver-related events; however, patients with CAP <248 dB/m had a tendency to develop decompensation[107]. In a study including 4,282 patients with chronic liver disease, CAP was not associated with liver-related events, the development of cancers other than HCC or cerebrovascular accident[108]. However, the median LSM in this study was low (6.1 kPa) and patient events were infrequent (1–2% during a median follow-up of 26 months), which might have limited the ability to detect an association. In order to further investigate the effects of hepatic steatosis on liver-related events and prognosis, future studies with large populations are needed, with adjustment for the aetiology of liver disease and fibrosis stage.

NASH clinical trial outcomes and design

High screen failure rates due to unmet histological inclusion criteria are a major problem in clinical trials in NASH; for example, in phase IIA and phase IIB trials, screen failure rates might be higher than 70%[109,110]. To reduce this high screen failure rate, we propose a two-step strategy (FIG. 2). As a first step, CAP should be performed on potential trial participants because CAP is a point-of-care test and is low cost. If the CAP value is above a certain threshold (which depends upon the MRI-PDFF inclusion criteria), the patient might be moved to the next step and undergo MRI-PDFF. In patients with CAP <288 dB/m, no further testing by MRI-PDFF is required and the patient can be excluded from the study due to the low likelihood of MRI-PDFF ≥5%. This step could lead to substantial cost savings and enrichment of the population that is likely to meet inclusion criteria. Many phase IIA trials have inclusion criteria for a baseline MRI-PDFF of ≥10%. For these studies, a CAP threshold of 306 dB/m could be utilized to decide which patients should be referred for a baseline MRI-PDFF. Those patients with a CAP value below the threshold will then be screen-failed, which will lead to considerable cost savings, by reducing unnecessary MRI examinations in patients who are unlikely to meet inclusion criteria. This two-step liver fat assessment and pre-screening strategy for enrolment in clinical trials in NASH helps reduce the screen failure rate (to <35%)[85,111] and reduces costs by reducing the requirement for upfront MRI-PDFF, as well as facilitating fast and streamlined recruitment of participants.

The histological assessment of outcomes is another problem in clinical trials in NASH. Histological response (NAS ≥2 points improvement with no worsening of fibrosis) or

histological fibrosis regression are used as a clinical outcome in early-phase clinical trials. However, repeat biopsies are necessary to the evaluation and biopsy has several limitations including invasiveness, sampling error, and only moderate intraobserver and interobserver reproducibility[12]. One advantage of non-invasive modalities is that repeat measurements are easy to obtain to assess changes in a disease condition. Since a 30% decline in MRI-PDFF relative to baseline is significantly associated with histological response and fibrosis regression, MRI-PDFF could be used as a surrogate marker of clinical trial outcomes. Based on these results, MRI-PDFF is now the preferred method for inclusion in phase I–IIB trials in NASH. Furthermore, MRI-PDFF is also a biomarker of treatment response in early-phase trials in NASH for therapies that have an anti-steatotic mechanism of action. The FDA permits the use of MRI-PDFF as the method for enrolment and evaluating therapeutic effect for early-phase clinical trials in NASH instead of liver biopsy, and MRI-PDFF is currently being used in some proof-of-concept clinical trials[110,111].

Conclusions

The degree of hepatic steatosis and its temporal change, as measured by quantitative modalities, are associated with disease progression and prognosis in NAFLD. Liver fat content in patients with NAFLD has been observed to decrease as cirrhosis develops; this phenomenon is sometimes referred to as burned-out NASH cirrhosis. Therefore, the clinical importance of changes in liver fat content should be assessed in accordance with the NAFLD disease status, particularly the degree of fibrosis. To achieve this goal, it will be necessary to evaluate patients who are stratified by baseline NAFLD status and liver fibrosis stage. In addition, the relationship between the values of non-invasive biomarkers of both liver fat and fibrosis should be examined, particularly in those who have co-existing advanced fibrosis (FIG. 3). In NAFLD without advanced fibrosis, a high liver fat level is associated with increased odds for fibrosis progression. On the other hand, in patients with NASH and advanced fibrosis, a lower liver fat level is associated with an increased incidence of liver-related events and poor prognosis. One advantage of non-invasive and quantitative methods is that repeat measurements are easy to obtain and they can evaluate temporal changes. In the studies investigating the association between changes in non-invasive assessment of liver fat and liver histology in NAFLD, the utility of a ≥30% decline in MRI-PDFF relative to baseline has been validated across multiple treatment trials[16]. In future studies, it will be necessary to examine if a larger improvement (that is, MRI-PDFF super-responder, ≥50% decline relative to baseline) will lead to increased rates of histological improvement and fibrosis regression as proposed in a 2020 paper[112].

A 30% decline in MRI-PDFF relative to baseline is a predictive factor for a histological response and fibrosis regression in patients with NAFLD without advanced fibrosis. However, it is unclear whether a 30% decline in MRI-PDFF relative to baseline could be applied for estimation of histological changes in patients with advanced fibrosis. Further investigation of this point is needed in future studies. The ultimate goal in NAFLD treatment is to avoid liver failure and liver-related complications, and to reduce mortality. However, the association between a 30% decline in MRI-PDFF relative to baseline and long-term clinical outcomes remains to be assessed. Therefore, further studies are needed to examine

if sustained reductions in liver fat will lead to improved survival in patients with stage 2 or stage 3 fibrosis with NASH.

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Key points

- Ultrasound-based modalities for the quantification of liver fat content have moderate diagnostic accuracy for the degree of hepatic steatosis and are useful as a pre-screen strategy in clinical trials in nonalcoholic steatohepatitis (NASH).
- Compared with other imaging modalities, MRI-derived proton density fat fraction (MRI-PDFF) has the highest diagnostic accuracy for quantification of liver fat content and is commonly used in trials in NASH.
- Increased liver fat content (MRI-PDFF ≥15%) is associated with increased odds of fibrosis progression in patients with NAFLD at an early stage of fibrosis.
- In patients with NAFLD, change in MRI-PDFF (≥30% decline relative to baseline) is associated with a histological response (NAFLD activity score ≥2 points improvement with no worsening of fibrosis) and fibrosis regression (reduction of one or more stages).
- Decreased liver fat content is associated with an increased incidence of liver-related events and poor prognosis in patients with NASH with advanced fibrosis.
- Liver fat content decreases in the setting of cirrhosis; therefore, the clinical importance of quantitative assessment of liver fat content and its change over time differs by NAFLD disease status and fibrosis severity.

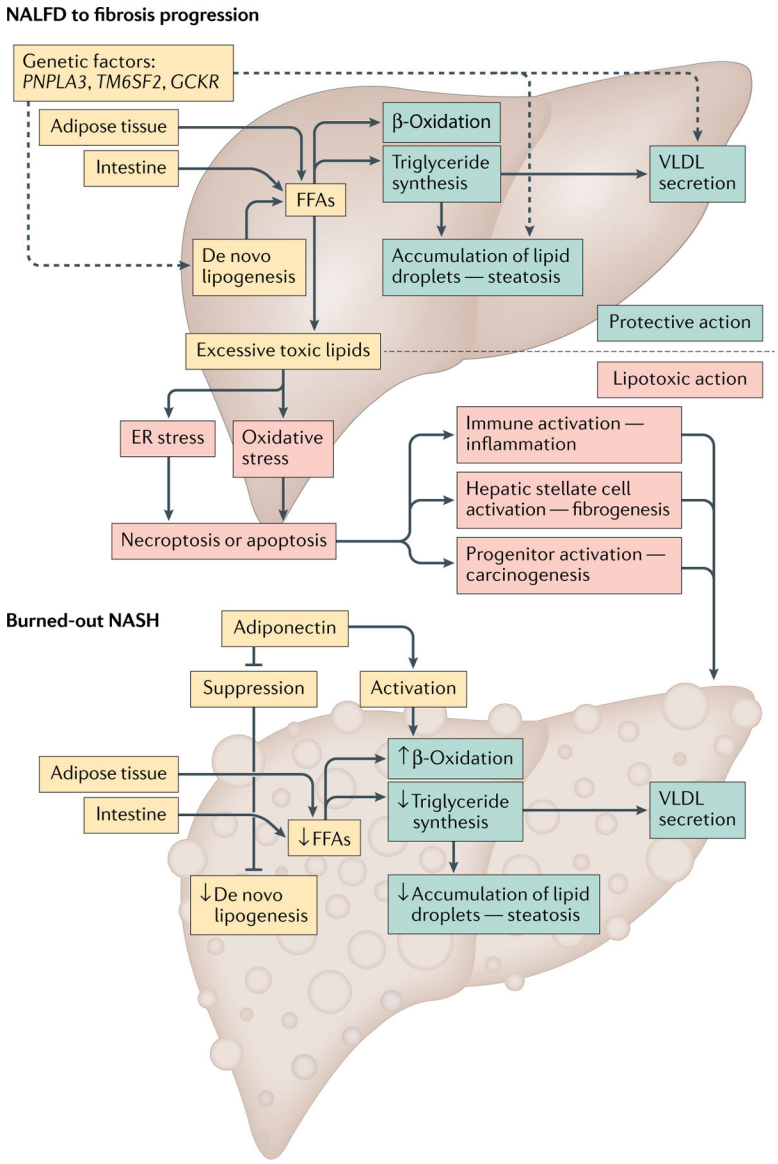

Fig. 1 |. Accumulation of lipids in the liver and disease progression in NAFLD.

In the liver, the main sources of free fatty acids (FFAs) are the influx of FFAs from adipose tissue, dietary FFAs and de novo lipogenesis. FFAs can enter the mitochondria and undergo β-oxidation to release energy. FFAs are also esterified to triglycerides, and triglycerides can be stored as lipid droplets or exported to blood as VLDL. In nonalcoholic fatty liver disease (NAFLD), lipid metabolism is disrupted: the influx of FFAs increases, β-oxidation and secretion of VLDL decrease. Consequently, the accumulation of intrahepatic triglycerides increases. When increased FFAs exceed the capacity of triglyceride synthesis and storage, lipotoxic species increase and these toxic lipids can cause cellular toxicity. Damaged hepatocytes release signals to promote the regeneration of hepatocytes, which ultimately can lead to inflammation, fibrosis and carcinogenesis. Genetic factors can influence NAFLD progression by increasing de novo lipogenesis and decreasing lipolysis and VLDL secretion. Paradoxically, liver fat content decreases as liver fibrosis increases to advanced fibrosis

or cirrhosis, known as ‘burned-out’ nonalcoholic steatohepatitis (NASH). Adiponectin acts directly on hepatocytes and has protective effects on NAFLD progression by increasing β-oxidation, decreasing fatty acid synthesis and improving insulin sensitivity. However, in burned-out NASH, adiponectin levels increase and elevated levels of adiponectin are associated with decreases in liver fat content. ER, endoplasmic reticulum.

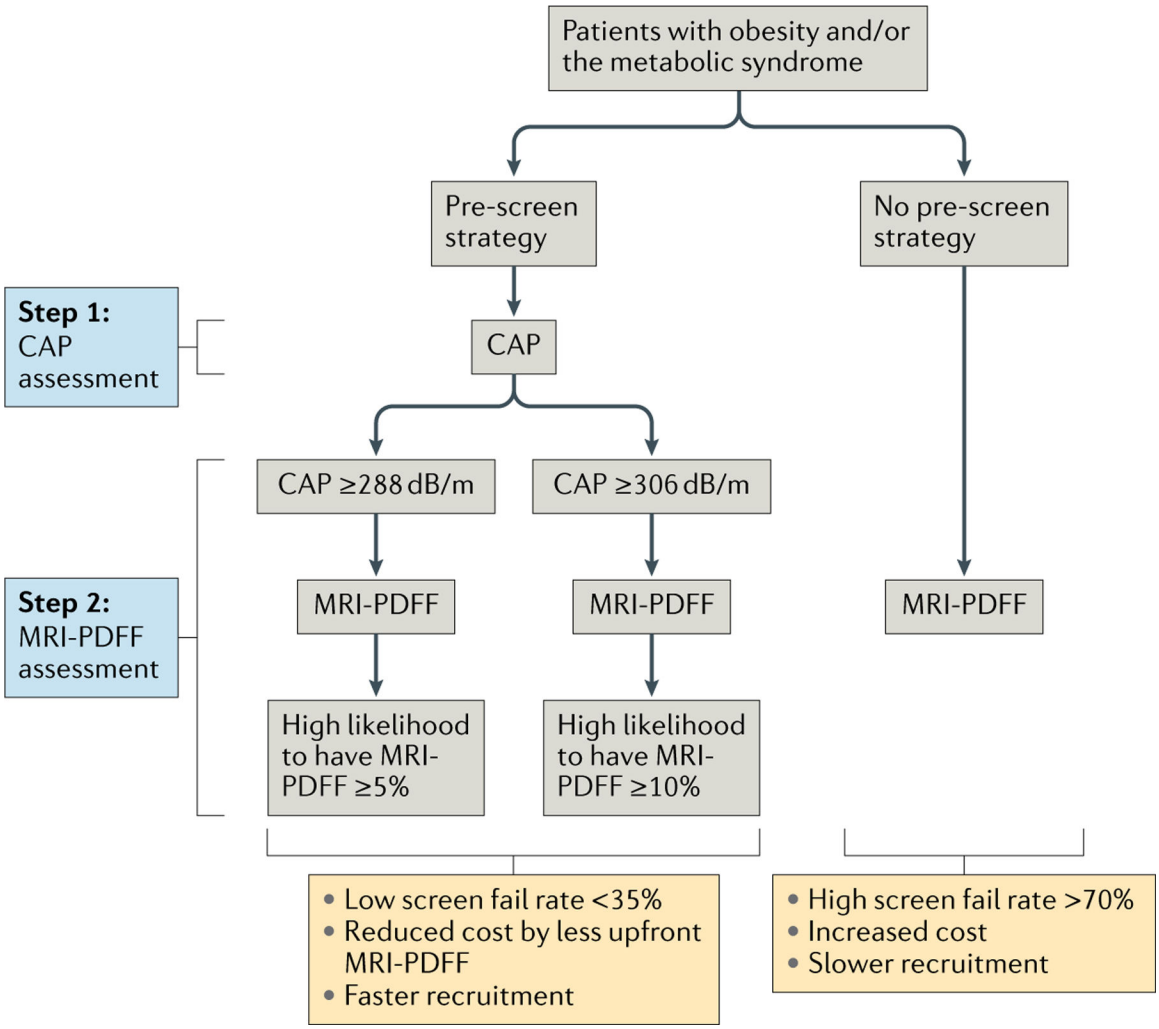

Fig. 2 |. Two-step liver fat content assessment to reduce screen failure rate in trials in NASH.
To reduce high screen failure rate in trials in nonalcoholic steatohepatitis (NASH), a two-step strategy is proposed. In the first step, controlled attenuation parameter (CAP) should be performed on potential trial participants with obesity and/or type 2 diabetes mellitus and/or the metabolic syndrome. If CAP falls above a certain threshold (depending upon the trial MRI-derived proton density fat fraction (MRI-PDFF) inclusion criteria), the patient can be moved to the next step and undergoes MRI-PDFF. Patients with CAP <288 dB/m can be excluded from the trial at this point due to the low likelihood of MRI-PDFF ≥5%. Patients with CAP ≥288 dB/m have a high likelihood of having MRI-PDFF ≥5% and patients with CAP ≥306 dB/m have a high likelihood of having MRI-PDFF ≥10%. Patients below the CAP threshold will be screen-failed, which will lead to large cost savings by reducing unnecessary MRI examinations, fast recruitment of trial participants and a reduced screen failure rate (<35%).

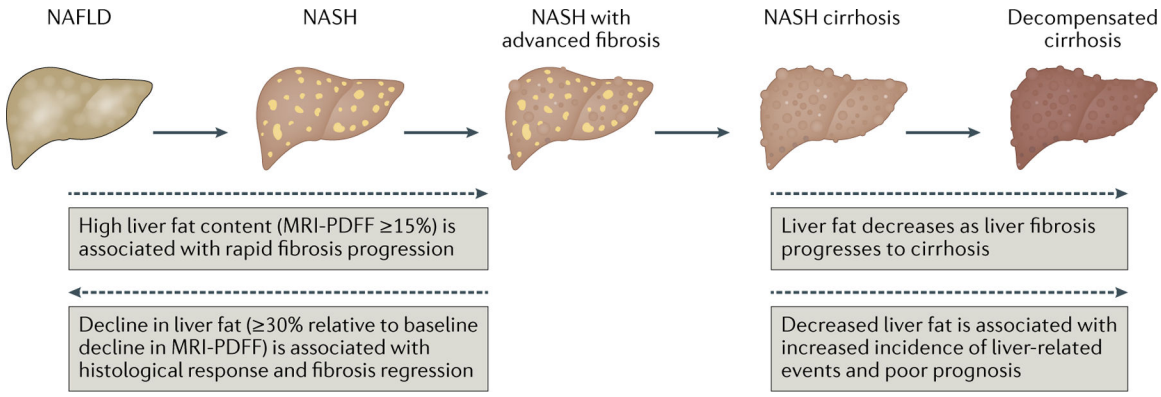

Fig. 3 |. Clinical importance of quantitative liver fat content assessment in NAFLD.

In nonalcoholic fatty liver disease (NAFLD), liver fat content decreases as liver fibrosis progresses to cirrhosis, which is known as burned-out nonalcoholic steatohepatitis (NASH). Therefore, the clinical importance of quantitative measurements of liver fat content varies depending upon NAFLD status. Patients should be stratified by fibrosis stage to investigate the association between quantitative measurements of liver fat content and NAFLD disease progression. In patients with NAFLD and NASH with early-stage fibrosis (without advanced fibrosis), high liver fat content (MRI-derived proton density fat fraction (MRI-PDFF) ≥15%) is associated with rapid fibrosis progression. Furthermore, a decline in liver fat content (≥30% decline in MRI-PDFF relative to baseline) is associated with a histological response (≥2 points improvement of NAFLD activity score with no worsening of fibrosis) and fibrosis regression (reduction of one or more stages). However, in patients with advanced disease, from advanced fibrosis to decompensated cirrhosis, decreased liver fat content is associated with an increased incidence of liver-related events and poor prognosis.

Table 1 |

Characteristics of each imaging modality for quantifying liver fat content

Imaging modality	Clinical utility	Limitations
Conventional B-mode ultrasonography	High availability; low cost	Not quantitative; low diagnostic accuracy for steatosis (20–30%)
CAP	Moderate accuracy for diagnosing steatosis; well validated	High rate of measurement failure; compatibility of M probe and XL probe; measurement on A-mode image
UGAP, ATT and ATI	Diagnostic accuracy is equivalent to that of CAP; measurement on ultrasonography B-mode images; lower frequency of measurement failure than CAP	Limited number of studies
QUS for fat fraction estimation	QUS approximates MRI-PDFF	Limited number of studies
MRI-PDFF	High accuracy for diagnosing steatosis; well validated	High cost; limited availability

ATI, attenuation imaging; ATT, attenuation coefficient; CAP, controlled attenuation parameter; MRI-PDFF, MRI-derived proton density fat fraction; QUS, quantitative ultrasonography; UGAP, ultrasound-guided attenuation parameter.

Table 2 |

Association between change in MRI-PDFF and histological change in patients with NAFLD

Study	Study population	Number of patients	Evaluation periods for histological change	Histological outcome	Association between MRI-PDFF change and histological outcome
Middleton et al.[86]	Patients with NASH treated with obeticholic acid or placebo	113	From baseline to 72 weeks	Improvement in steatosis grade	Absolute change of −5.1% was optimal threshold for steatosis improvement at 90% specificity
				Worsening of steatosis grade	Absolute change of 5.6% was optimal threshold for steatosis worsening at 90% specificity
Middleton et al.[87]	Children with NASH treated with cysteamine bitartrate delayed release or placebo	169	From baseline to 54 weeks	Improvement in steatosis grade Worsening of steatosis grade	Absolute change of −11.0% was optimal threshold for steatosis improvement at 90% specificity Absolute change of 5.5% was optimal threshold for steatosis worsening at 90% specificity
Loomba et al.[88]	Patients with NASH treated with selonsertib or placebo	72	From baseline to 24 weeks	Improvement in steatosis grade	−20.2% relative decline was observed in patients with steatosis improvement (versus −0.8% in patients without steatosis improvement)
Patel et al.[89]	Patients with NASH treated with ezetimibe or placebo	35	From baseline to 24 weeks	Histological response[a]	−29.3% relative decline was observed in patients with histological response (versus 2.0% in patients without histological response)
Jayakumar et al.[90]	Patients with NASH treated with selonsertib and/or simtuzumab	65	From baseline to 24 weeks	Histological response[a]	≥25% relative decline was optimal threshold for ≥histological response (50% sensitivity, 81% specificity, 38% PPV and 92% NPV)
Loomba et al.[91]	Patients with NASH treated with obeticholic acid or placebo	78	From baseline to 72 weeks	Histological response[a]	≥30% relative decline was optimal threshold for histological response. Patients with ≥30% relative decline achieved 50% histological response with OR 4.86 (versus 19% in patients without ≥30% relative decline)
Harrison et al.[92]	Patients treated with resmetirom	73	From baseline to 12 weeks	Histological response[a]	Patients with relative decline ≥30% achieved 37% histological response (versus 4% in patients without relative decline ≥30%)
Bril et al.[93]	Patients treated with pioglitazone or placebo	121	From baseline to 6 or 18 months[b]	Histological response[a]	Reduction in steatosis was not associated with histological response in pioglitazone group or placebo group, but a significant association was observed in the two groups combined
Stine et al.[16]	Meta-analysis including seven studies	346	NA	≥2 points reduction in NAS, at least 1 point reduction in lobular inflammation or ballooning	Patients with ≥30% relative decline achieved 51% histological outcome with OR 6.98 (versus 14% in patients without ≥30% relative decline)
Tamaki et al.[17]	Patients received a paired biopsy with contemporaneous MRI-PDFF assessment	100	Median interval between paired biopsies: 1.4 years	Fibrosis regression (reduction of one or more stages)	≥30% relative decline in MRI-PDFF was an independent predictor of fibrosis regression with an adjusted OR 6.46

MRI-PDFF, MRI-derived proton density fat fraction; NA, not applicable; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value.

[a]Histological response was defined as reduction in NAS of ≥2 points with no worsening of fibrosis.

[b]Steatosis was assessed by ^{1}H-magnetic resonance spectroscopy.

Table 3 |

Association between liver fat content and liver-related outcomes and mortality

Study	Number of patients	Distribution of fibrosis in the study population[a]	Study design	Aetiology	Liver lipid evaluation method	Outcome	Main result
Younossi et al.[96]	209	Fibrosis stages 0–4	Longitudinal study	NAFLD	Biopsy	Liver-related mortality	Steatosis grade was not associated with liver-related mortality
Angulo et al.[97]	619	Fibrosis stages 0–4	Longitudinal study	NAFLD	Biopsy	Liver-related events and death	Steatosis grade was not associated with liver-related events or mortality
Hagström et al.[98]	646	Fibrosis stages 0–4	Longitudinal study	NAFLD	Biopsy	Liver-related events and death	Steatosis grade was not associated with liver-related events or mortality
Vilar-Gomez et al.[102]	458	Fibrosis stages 3 and 4	Longitudinal study	NAFLD	Biopsy	Liver-related events and death	Steatosis grade <33% was associated with higher incidence of liver-related events and death independent of presence of cirrhosis
Ajmera et al.[15]	95	Fibrosis stages 0–4	Longitudinal study	NAFLD	MRI-PDFF	Fibrosis progression	MRI-PDFF ≥15% was associated with fibrosis progression in patients with no fibrosis
Abdelaziz et al.[103]	130	Compensated cirrhosis	Cross-sectional study	HCV	CAP	Presence of HCC	CAP value was significantly lower in patients with HCC (209 dB/m versus 259 dB/m)
Izumi et al.[104]	1,054	All levels of liver stiffness	Cross-sectional study	HCV, HBV or NAFLD	CAP	Presence of HCC	CAP value ≤221 dB/m in HCV and ≤265 dB/m in NAFLD were associated with presence of HCC
Semmler et al.[105]	261	78.5% had cirrhosis assessed by VCTE	Cross-sectional study	Mixed	CAP	Presence of notable portal hypertension (HVPG ≥10 mmHg)	Higher CAP was an independent protective factor for the presence of significant portal hypertension
Mendoza et al.[106]	272	Patients with obesity and with advanced fibrosis (≥10 kPa by VCTE)	Longitudinal study	Mixed	CAP	Liver-related events	CAP ≥220 dB/m was associated with a reduced risk of decompensation with HR 0.043
Scheiner et al.[107]	430	Patients with advanced fibrosis including decompensated cirrhosis (≥10 kPa by VCTE)	Longitudinal study	Mixed	CAP	Liver-related events	CAP did not predict the development of first and further liver-related events but had a tendency to predict the development of first decompensation in patients with CAP <248 dB/m
Liu et al.[108]	4,282	All levels of liver stiffness (median LSM 6.1 kPa)	Longitudinal study	Mixed	CAP	Liver-related events, non-HCC cancer and cerebrovascular accident	CAP was not associated with liver-related events, non-HCC cancer or cerebrovascular accident

CAP, controlled attenuation parameter; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HVPG, hepatic venous pressure gradient; LSM, liver stiffness measurement; MRI-PDFF, MRI-derived proton density fat fraction; NAFLD, nonalcoholic fatty liver disease; VCTE, vibration-controlled transient elastography.

[a]Fibrosis stage was defined as follows: stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal or periportal fibrosis; stage 3, bridging fibrosis; stage 4, cirrhosis.