

## Review

# What's new in non-alcoholic fatty liver disease?

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## ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide, with an estimated prevalence of 25% in the Western World. NAFLD is a broad spectrum of disease states and while most people with NAFLD do not have progressive disease, 10–20% of patients develop histological features of inflammation (non-alcoholic steatohepatitis), fibrosis, cirrhosis and its complications. Despite this large disease burden of significant clinical impact, most people living with NAFLD are undiagnosed, disease course prediction is imprecise and there are no treatments licensed for this condition. In this review, we discuss some of the recent developments in NAFLD, focusing on disease definition and diagnosis, risk stratification and treatments.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently defined as the accumulation of excessive fat in the liver in the absence of secondary causes or other liver disease aetiologies. It is the most common cause of liver disease worldwide and is closely linked to type II diabetes (T2DM) and obesity with recent meta-analyses estimating the prevalence at approximately 25% in the Western World. NAFLD is a broad spectrum of disease states and most people with NAFLD have simple steatosis with low risk of progression and low risk of liver-related mortality or morbidity. The presence of T2DM can accelerate disease progression and approximately 10%–20% of patients can develop the progressive, inflammatory form: non-alcoholic steatohepatitis (NASH), characterised by lobular inflammation and hepatocyte ballooning which is associated with the development of fibrosis, cirrhosis and its complications including liver failure and hepatocellular carcinoma (HCC).<sup>1</sup> Despite this large disease burden of significant clinical impact, most people living with NAFLD are undiagnosed,<sup>2</sup>

## Key messages

- ⇒ Non-alcoholic fatty liver disease (NAFLD) is a common condition associated with significant liver-, cardiovascular- and cancer-related mortality.
- ⇒ The proposed terminology metabolic-associated fatty liver disease acknowledges the potential for more than one aetiology of liver disease, but may also select patients with metabolic factors that increase the risk of liver disease progression, and therefore identifies patients with poorer prognosis compared with patients with NAFLD.
- ⇒ Risk stratification for significant or advanced fibrosis in NAFLD currently involves a combination of calculated scores (such as the Fibrosis-4 index or NAFLD fibrosis score), biomarkers of fibrosis (such as the Enhanced Liver Fibrosis test) and transient elastography.
- ⇒ Large-scale initiatives are underway to develop and validate biomarkers to detect fibrosis.
- ⇒ Lifestyle and behaviour change remains the cornerstone of treatment in NAFLD.
- ⇒ There are no drugs licensed for non-alcoholic steatohepatitis, although several drugs are currently in advanced phase trials.

disease course prediction is imprecise and there are no treatments licensed for this condition. This review will highlight some of the recent developments in NAFLD, focusing on disease definition and diagnosis, risk stratification and treatments.

## Is NAFLD now MAFLD?

Patients may have more than one aetiology affecting the liver at the same time, but the current definition of NAFLD requires that other causes of liver disease are excluded. Therefore, the increasingly described synergistic effect of multiple aetiologies on disease progression is not acknowledged, large numbers of patients



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are excluded from clinical studies in this field, including therapeutic trials, and patients' metabolic risk may not be recognised or addressed. Recognising this close association with metabolic risk, an international consensus has proposed the term metabolic-associated fatty liver disease (MAFLD)<sup>3</sup> as the presence of hepatic steatosis plus one of the following: overweight/obese, T2DM or evidence of two or more features of metabolic dysfunction.<sup>4</sup> While this reflects the real-world disease burden, by selecting patients with metabolic factors that are also risk factors for liver disease progression, the new definition identifies patients with poorer prognosis compared with patients with NAFLD.<sup>5</sup> Therefore, a change in definition will have implications for clinical trial design and biomarker discovery and may impact decisions around the treatment of comorbidities.

#### Advances in epidemiology and natural history

The estimated global prevalence of NAFLD is approximately 25%,<sup>6</sup> however population-level data from four European primary care databases of over 18 million patients found that only 0.7% of patients had a recorded diagnosis of either NAFLD or NASH.<sup>2</sup> This is in keeping with another primary care study, which found a prevalence of 1.85%,<sup>7</sup> highlighting a significant diagnosis gap and under-recording. Steatosis is also prevalent in young people, with a recent UK study finding that 20% of young people aged 22–26 years had steatosis, and 1 in 40 had fibrosis, on transient elastography.<sup>8</sup>

NASH is closely associated with the development of fibrosis, but, importantly, it is the stage of fibrosis, rather than the grade of ballooning or inflammation that predicts progression and associated poor outcomes, including liver-related mortality,<sup>9,10</sup> notwithstanding the risk of lead time bias in such studies. The clinical challenge is therefore to identify those patients with fibrosis due to NASH, who are at higher risk of disease progression. However, NASH is a histological diagnosis and there are no clinically useful tools to distinguish NASH from simple steatosis.

NAFLD is associated with morbidity and mortality related to other organ systems, even more commonly than with liver disease. People with pre-diabetes and T2DM who also have NAFLD have higher rates of complications of diabetes; renal failure, diabetes progression and cardiovascular disease (CVD).<sup>11,12</sup> The link between established cardiometabolic risk factors and NAFLD is supported by epidemiological<sup>13,14</sup> as well as mechanistic data,<sup>15</sup> and although there is debate as to whether liver disease poses added, independent risk for CVD,<sup>16</sup> patients with NAFLD are at risk of CVD and currently available risk stratification tools such as QRISK3<sup>17</sup> should be used in these patients.

NAFLD is associated with higher cancer incidence rate ratio (2.0; 95% CI 1.5 to 2.9) in 4772 patients compared with 14441 sex-matched and age-matched non-obese controls, with the greatest increase in

HCC,<sup>17</sup> which persists after controlling for diabetes.<sup>18</sup> In a recent meta-analysis of 61 studies, including 94636 individuals globally, patients who developed HCC on a background of NAFLD were older, had higher body mass index and were more likely to have metabolic comorbidities compared with HCC due to other causes.<sup>19</sup> A total of 38.5% of patients with HCC in the context of NAFLD presented with HCC in a non-cirrhotic liver, compared with 14.6% for HCC due to other causes. In this study, a significantly lower proportion of patients with NAFLD-HCC underwent surveillance; 32.8% compared with 55.7% for HCC due to other causes.

NAFLD also impacts patients' quality of life (QoL), especially in the physical subdomain, with the presence of fatigue and excessive daytime sleepiness being significantly associated with worse health-related QoL.<sup>20,21</sup> Individuals with NAFLD, even without advanced fibrosis, have lower QoL than controls.<sup>22</sup> However, there is a significant association with disease severity: patients with NASH have reduced health-related QoL compared with those with NAFLD,<sup>23</sup> and patients with NASH-cirrhosis have lower health-related QoL than patients with non-cirrhotic NASH.<sup>24</sup>

#### Genetic risk in NAFLD

Established risk factors for NASH include the presence of insulin resistance and metabolic syndrome, age, sex, diet and ethnicity. Genetic predisposition is believed to play a role, with heritability estimates ranging from 20% to 70%.<sup>25</sup> Genome-wide association studies (GWAS) have identified the PNPLA3 and TM6SF2 genes to be associated with steatosis,<sup>26</sup> as well as grade of NASH, stage of fibrosis<sup>27,28</sup> and in the case of PNPLA3, development of HCC.<sup>29</sup> A recent large histology-based NAFLD GWAS showed that a missense variant in the gene PYGO1, which encodes a transcription factor that contributes to the Wnt signalling pathway, had close to genome-wide significance for steatosis, and an intronic variant near the LEPR gene, which encodes for a leptin receptor, had genome-wide significance for NASH.<sup>30</sup> The absence of biomarkers for NASH poses challenges for conducting GWAS in populations who have not had a liver biopsy. An MRI-based corrected T1 (cT1) score approximates to the severity of NASH and liver fibrosis and this, in combination with elevated liver enzymes, is significantly associated with variants in metal ion transporter genes in SLC30A10 and SLC39A8.<sup>31</sup>

The challenge for the field will be to translate these genomic discoveries into clinical care—whether in drug target identification, improved diagnostics or linking genomic risk with targeted therapy. A recent exome-wide association study found that a loss-of-function variant in HSD17B13 was associated with lower rates of NASH, although the variant was not associated with simple steatosis, suggesting that HSD17B13 may be involved in more clinically advanced stages

of NAFLD.<sup>32</sup> This discovery has led to the development of ARO-HSD, an RNA-interfering therapeutic that selectively targets HSD17B13 mRNA in hepatocytes with early signals that the intervention reduces HSD17B13 expression in phase 1 and 2a clinical trials (NCT04565717; NCT04202354).<sup>33</sup>

### Clinical tools for risk stratification

The diagnosis of NAFLD is usually made after excess liver fat is demonstrated by ultrasound, MRI or other modality. The clinical challenge is then to identify the subset of patients with NASH and fibrosis, who are at higher risk of disease progression. Liver biopsy may be the gold standard investigation for determining fibrosis stage in NAFLD, but it is invasive, costly, carries a risk of complications and, probably most importantly, is subject to sampling and inter- and intra-observer variability.<sup>34</sup> However, clinical assessment or serum transaminase levels alone are inadequate for detecting fibrosis or identifying patients with a more aggressive disease course. Hence, non-invasive liver tests have been developed to give a proxy estimate of the risk of fibrosis in patients with a clinical diagnosis of NAFLD. Focused hypothesis-driven research as well as large-scale initiatives to develop and validate biomarkers, such as the NIMBLE Programme in the USA<sup>35</sup> and the LITMUS Programme in the European Union,<sup>36</sup> are underway and will advance research and practice in this area.

The simplest tests are scores calculated from the results of routinely collected clinical parameters such as the Fibrosis-4 (FIB-4) index or the NAFLD fibrosis score. Others are biomarkers of fibrosis such as Enhanced Liver Fibrosis (ELF) test or estimate physical properties of the liver by transient elastography or acoustic radiation force impulse. The utility of these tests is to stratify patients according to the likelihood of significant (stages 2–4) or advanced (stages 3–4) fibrosis. The Camden and Islington NAFLD Pathway uses two tests sequentially; FIB-4 followed by ELF for patients in the indeterminate range to triage referrals from primary care to specialist hepatology services.<sup>37</sup> In a prospective evaluation, the pathway reduced the proportion of referrals for patients with stages 0–2 fibrosis (termed ‘unnecessary referrals’) by 82%, and led to a fivefold increase in referrals of cases of advanced fibrosis and cirrhosis and threefold increase in detection of cases of cirrhosis.<sup>37</sup> A similar approach using blood test-based scores and transient elastography is recommended by European guidelines.<sup>9</sup>

Despite evidence of utility in improving service provision, the efficacy of many of these non-invasive tests to identify fibrosis—particularly in unselected populations where the prevalence of advanced fibrosis is low, such as primary care—remains uncertain.<sup>38</sup> A recent population-level study in Sweden found that several non-invasive liver tests provided only modest predictive ability for the development of serious liver

disease at 5 and 10 years.<sup>38</sup> For instance, the area under the receiver operating characteristic (AUROC) for 5-year outcomes was 0.74 for FIB-4 and 0.52 for NAFLD fibrosis score.<sup>38</sup> Interestingly, all five of the scoring systems evaluated had lower predictive abilities in women compared with men,<sup>38</sup> suggesting that future risk-stratification algorithms may benefit from including gender. A UK-based study found that non-invasive scores were less accurate in patients of South Asian ethnicity, irrespective of metabolic parameters.<sup>39</sup> Conversely, there was no significant difference in accuracy of non-invasive scores between white patients and Asian patients (predominantly residents in East Asia) enrolled in the STELLAR 3 and 4 trials, although these patients were likely to have been preselected using the same scores for active and advanced NASH as the key inclusion criteria for the trials.<sup>40</sup>

A current imperative for biomarker discovery is to reduce the high screen-fail rates in clinical trials in NASH that relate to histological diagnosis, although this is multifactorial. The Fibroscan-AST score combines transient elastography (Fibroscan) measurements and serum aspartate aminotransferase (AST) to identify patients with significant fibrosis and NASH, with an AUROC of 0.8.<sup>41</sup> NIS4, a blood-based panel of four microRNA species, has comparable accuracy with AUROC of 0.8.<sup>42</sup> While these tests have been developed to identify the clinical trial population, much work is needed to determine whether any non-invasive strategy (and if so, which one) could replace histology as an inclusion criterion or endpoint in clinical trials in NASH.

MRI-PDFF (protein density fat fraction) and proton magnetic resonance spectroscopy are accurate measures of hepatic steatosis,<sup>43</sup> and magnetic resonance elastography can be used to characterise fibrosis and estimate fibrosis stage.<sup>44 45</sup> Although MRI alone does not differentiate NASH (ballooning or inflammation) from simple steatosis,<sup>46</sup> iron-cT1 MRI is able to identify patients with liver inflammation and fibrosis<sup>47</sup> and correlates with histological features of NASH.<sup>48</sup> PDFF is used as a non-invasive endpoint in early phase drug development, and recent evidence that changes in PDFF after 12 weeks’ therapy predicted response to Resmetirom in patients with NASH offers the potential for on-treatment prediction as a tool to personalise drug therapy.<sup>49</sup>

### Management

#### Lifestyle intervention

Lifestyle and behaviour change is the cornerstone of treatment in NAFLD and international and UK guidelines recommend weight loss through a combination of diet and exercise.<sup>9 50</sup> This advice is communicated directly to patients in the recently published EASL patient guideline.<sup>51</sup> Weight loss of  $\geq 5\%$  is associated with improvement in liver fat and liver enzymes,<sup>52</sup> and weight loss of  $\geq 7\%$ , and particularly  $\geq 10\%$ , is

associated with NASH resolution and fibrosis improvement.<sup>53</sup>

To determine the effect of different diets on liver fat, fibrosis and function, a recent systematic review and meta-analysis of randomised control trials (RCTs) of dietary interventions without exercise or physical activity found that a Mediterranean diet reduced liver fat in patients with NAFLD, even without calorie restriction.<sup>54</sup> The same analysis showed that a hypocaloric diet favouring unsaturated fatty acids reduced liver transaminases.<sup>54</sup> Meanwhile, a recent RCT compared intermittent calorie restriction (the 5:2 diet) and a low-carbohydrate high-fat diet (LCHF) with generalised lifestyle advice and found that both diets were superior to lifestyle advice alone in reducing steatosis (absolute reduction:  $-6.1\%$  and  $-7.2\%$ , respectively, vs  $-3.6\%$ ) and body weight ( $-7.4$  kg and  $-7.3$  kg, respectively, vs  $-2.5$  kg).<sup>55</sup> While there was no difference between the two diets for steatosis or weight change, liver stiffness improved in the 5:2 diet, but not the LCHF diet, and the 5:2 diet was associated with reduced low-density lipoprotein (LDL) levels, while being tolerated to a higher degree.<sup>55</sup>

There is also attention on causative dietary constituents, such as fructose.<sup>56</sup> Fructose consumption is a known risk factor for the development of NAFLD and increasing evidence highlights fructose as a driver of NAFLD pathogenesis.<sup>57</sup> The majority of fructose is cleared on first pass through the hepatic circulation by phosphorylation via the ketohexokinase (KHK) enzyme, thus a recent study evaluated the response to KHK inhibition in mice, human co-cultures and human liver tissue.<sup>58</sup> KHK inhibition was found to improve steatosis, fibrosis and inflammation, identifying a potential therapeutic target for the future.<sup>58</sup>

The EASL patient guideline recommends that patients with NAFLD undertake at least 150 minutes of moderate intensity physical exercise per week, including a combination of aerobic and resistance training.<sup>51</sup> Evidence from meta-analyses demonstrates that physical activity reduces intrahepatic lipid content and markers of hepatocellular injury,<sup>59 60</sup> even in the absence of weight loss.<sup>59</sup> In general, studies have not found a difference in benefit between aerobic and resistance exercise.<sup>59 61</sup>

Bariatric or metabolic surgery results in significant weight loss and improvement in metabolic parameters. In a large, propensity matched cohort of 2057 patients, those who underwent bariatric surgery had a lower incidence of new onset NASH (6% vs 10%, adjusted OR 0.52) and HCC (0.05% vs 0.34%) over a median follow-up of 7.1 years, compared with morbidly obese patients who did not undergo surgery.<sup>62</sup> In 180 patients with NASH followed for 5 years after bariatric surgery, NASH resolved, without worsening fibrosis, in 84% of patients, fibrosis decreased in 70% of patients, and the reduction was progressive, beginning during the first year and continuing through 5 years.<sup>63</sup> RCT data are

currently unavailable (though studies are ongoing) to support the use of surgery as a treatment for NASH, but only a small proportion of all patients eligible for surgery are referred and surgery should be considered for established indications. However, the stage of liver disease should be assessed and if advanced fibrosis cannot be ruled out, specialist assessment to look for features of cirrhosis, portal hypertension and decompensation should be sought to determine safety of surgery.

#### Cardiovascular risk management

Given the association between NAFLD and CVD, clinicians should aim to stratify cardiovascular risk for all patients with NAFLD using currently available tools such as QRISK3. Optimising these risk factors may include management of blood pressure, diabetes and lipids, as well as lifestyle interventions to achieve weight loss and smoking cessation. These issues support a multidisciplinary approach to management, as demonstrated in a recent study where a team involving hepatologists, endocrinologists, and allied healthcare professionals including diet and lifestyle experts, was found to be clinically effective in managing a cohort of patients with NAFLD.<sup>64</sup>

#### Pharmacological treatment

Currently, there are no drugs licensed for NASH and some clinicians may consider off-license treatment with vitamin E or Pioglitazone based on the results of the PIVENS trial<sup>65</sup> and NICE guidance. However, many drugs are in the development pipeline and patients may wish to participate in clinical trials. For later-stage clinical trials, regulators have mandated histological inclusion criteria and endpoints (resolution of NASH with no worsening of fibrosis and/or improvement in fibrosis by one stage or more with no worsening of NASH) usually after 12–18 months' treatment, with longer term clinical endpoints needed for full registration.

A significant number of trials have not shown efficacy in high-profile late phase studies; including trials of Elafibrinor, Cenicriviroc, Simtuzumab, Selonsertib, Volixibat and most recently Aldafermin. Possible explanations include drug selection, heterogeneity of NASH, trial design and high placebo response rates (of 25% for improvement in NAFLD disease activity score (NAS) by 2 or more points in a recent meta-analysis<sup>66</sup>). The time frames for histology may also be too short to establish fibrosis regression, particularly in the clinically relevant advanced stages (F3–F4).

Several drugs are currently in phase III trials. Obeticholic acid, a farnesoid X receptor agonist involved in regulation of bile acids and metabolism, showed a positive signal in a phase II study, and interim analysis in the phase III trial (n=931) demonstrated a significant improvement in fibrosis with Obeticholic acid 25 mg per day in patients with NASH and significant

fibrosis.<sup>67</sup> While the secondary endpoint of histological improvement of features of NASH and improvement in NAS was not met, a post hoc analysis showed significantly higher rate of NASH resolution measured by pathologist assessment alone in the Obeticholic acid 25 mg group compared with placebo.<sup>67</sup> However, at a dose of 25 mg, 51% of patients reported pruritus, with severe intensity in 28%, and LDL cholesterol increased at both 10 mg and 25 mg doses, with 380 patients starting statin therapy during the study.<sup>67</sup>

PPARs (peroxisome proliferator-activated receptors) are nuclear receptors with key regulatory functions in metabolism, inflammation and fibrogenesis. In a phase IIb trial of 247 patients, Lanifibranor, a pan-PPAR agonist, led to an improvement of NASH, measured by a decrease of at least 2 points in the SAF-A score (the activity part of the Steatosis, Activity, Fibrosis scoring system) without worsening of fibrosis, compared with placebo (55% vs 33%).<sup>68</sup>

Resmetirom is a selective thyroid hormone receptor- $\beta$  agonist that increases hepatic fat metabolism and reduces lipotoxicity.<sup>69</sup> The phase II trial (n=125) demonstrated a reduction in hepatic fat after 12 weeks (-32.9% vs -10.4%) and 36 weeks (-37.3% vs -8.5%) of treatment in patients with NASH<sup>69</sup> and phase III trials are under way.

The phase 2 LEAN Study (n=52) evaluated the use of Liraglutide versus placebo in NASH and demonstrated greater weight loss (5.5% vs 0.7%) and NASH resolution (39% vs 9%) in those receiving Liraglutide.<sup>70</sup> However, participants had regained significant weight 3 months after ceasing treatment. More recently, Semaglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist with greater metabolic effects, was evaluated in 320 individuals with NASH and demonstrated 12.5% weight loss at highest dose compared with <1% weight loss in placebo group, which corresponded to 59% and 17% resolution of NASH, respectively, on follow-up biopsy, but without any significant improvement in fibrosis.<sup>71</sup>

A number of drugs that offer a beneficial metabolic profile are being developed or are already licensed for people with T2DM. In post hoc analysis of a phase II trial in people with T2DM (n=316), Tirzepatide (a dual glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptor agonist) at high dose (15 mg once weekly) for 28 weeks led to a significant reduction in NASH-related biomarkers (alanine aminotransferase (ALT), AST, K-18 and Pro-C3),<sup>72</sup> which has led to a separate phase 2 study in NASH (NCT04166773). Empagliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, reduced hepatic fat content by 1.8 percentage points.<sup>73</sup>

In this busy development landscape, a clear future treatment strategy has yet to emerge. Important questions include whether patients will be selected for therapy based on histological or non-invasive parameters, at what stage of disease patients should receive

therapy and whether combination therapy is superior to a single agent. Further questions such as on-treatment predictors of response, duration of therapy, and stopping rules will require extensive study and will depend on biomarker discovery. Use of drugs with metabolic benefit may be advantageous, but if drugs are licensed for comorbid conditions such as T2DM, some at-risk patients may take these for indications other than NASH.

## CONCLUSIONS

NAFLD is a common condition associated with significant liver-, cardiovascular- and cancer-related morbidity. Recent advances have focused on improving our understanding of epidemiology, diagnosis and treatments. As our understanding progresses, novel biomarkers and treatment strategies have begun to emerge. The challenge to the clinical community is how and in which patients to implement these advances and how their effectiveness will be measured. Moreover, these new advances will be implemented on the backdrop of the evolving treatment landscapes for the many comorbid conditions that patients with NASH live with.

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