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MANAGEMENT OF ENDOCRINE DISEASE

Non-alcoholic fatty liver disease: a multidisciplinary approach towards a cardiometabolic liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a growing health problem with a global prevalence of over 25% and prevalence rates of over 60% in high-risk populations. It is considered the hepatic component of the metabolic syndrome and is associated with an increased risk of the development of various liver-associated and cardiometabolic complications. Given the complexity of NAFLD and associated comorbidities and complications, treatment requires interventions from a variety of different healthcare specialties. However, many clinicians are currently insufficiently aware of the potential harm and severity of NAFLD and associated comorbidities, complications and the steps that should be taken when NAFLD is suspected. Recognizing which patients suffer from non-progressive simple steatosis, metabolically active NASH with high risk of developing cardiovascular disease and which patients have a high risk of developing cirrhosis and hepatocellular carcinoma is important. Unfortunately, this can be difficult and guidelines towards the optimal diagnostic and therapeutic approach are ambivalent. Here we review the pathogenesis, diagnostics and treatment of NAFLD and discuss how multidisciplinary care path development could move forward.

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Introduction

Worldwide, the amount of people leading a so-called 'Western lifestyle', an unhealthy high-caloric diet and only little exercise, has increased tremendously. Associated with this 'Western lifestyle' are obesity and the metabolic syndrome, a term used for the coexistence of an increased abdominal fat mass, hyperglycaemia, hypertension and dyslipidaemia. Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic component of metabolic syndrome (1, 2). NAFLD is defined by accumulation of intracellular fat in >5% of hepatocytes on imaging or histology, in the absence of other causes of hepatic steatosis such as excessive alcohol intake, certain metabolic conditions or drug use (3).

In concert with the increase in prevalence rates of obesity and metabolic syndrome, the prevalence of NAFLD has increased dramatically to over 25% of the population worldwide (4). In high-risk populations, like patients with type 2 diabetes mellitus (T2DM), prevalence rates are even estimated to be over 60% (5). The high prevalence of NAFLD and the associated complications and comorbidities, including T2DM and cardiovascular disease, result in a large burden on healthcare, associated

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costs, a reduction of quality of life and increased mortality rates (3, 6, 7, 8).

NAFLD encompasses a spectrum of disease stages. It varies from simple hepatic steatosis, also known as non-alcoholic fatty liver (NAFL), to steatohepatitis (NASH) and development of fibrosis and can eventually progress to cirrhosis and hepatocellular carcinoma (HCC) (Fig. 1) (6). Although NAFLD is very common, only a part of the patients with hepatic steatosis will develop advanced stage liver disease. However, when NAFLD progresses to a more severe liver disease, potential lethal complications like ascites, oesophageal varices, hepatic encephalopathy, HCC and liver failure may arise. It is important to differentiate between patients with non-progressive simple steatosis and patients with metabolically active NASH with high risk of developing cardiovascular disease or those with an increased risk of developing cirrhosis and HCC. Unfortunately, recognizing these at-risk patients can be difficult and guidelines on the optimal diagnostic and therapeutic approach are ambivalent. As a result, many clinicians are currently insufficiently aware of the steps that should be taken when NAFLD is suspected or newly diagnosed. A fundamental aspect of this problem is the paucity of a common healthcare path for NAFLD covering the complexity and multidisciplinary character of this potential harmful disease (9).

Here we review the pathogenesis, diagnostics and treatment of NAFLD and aim to provide a clear overview of the diagnostic options, clinical care strategies and recommendations for the development of a multidisciplinary care path.

Pathogenesis

The pathogenesis of NAFLD is complex. We give a graphical overview in Fig. 2. For extensive detail, we refer to excellent recent reviews (2, 4, 10).

In brief, an interaction of environmental factors, diet and genetics results in alterations of multiple factors and pathways of glucose and lipid metabolism that constitute vicious circles, leading to progressive stages of NAFLD. Centrally stands insulin resistance, which causes an increased flux of circulating free fatty acids (FFAs) to the liver, through reduced insulin-mediated suppression of lipolysis in adipose tissue (11, 12, 13). These FFAs are stored as triglycerides in lipid droplets, reducing hepatic insulin sensitivity and consequently increasing hepatic gluconeogenesis, which results in hyperglycaemia and intrahepatic conversion of glucose to FFAs (14). Meanwhile, high plasma insulin levels increase *de novo* lipogenesis, producing even more triglycerides and further

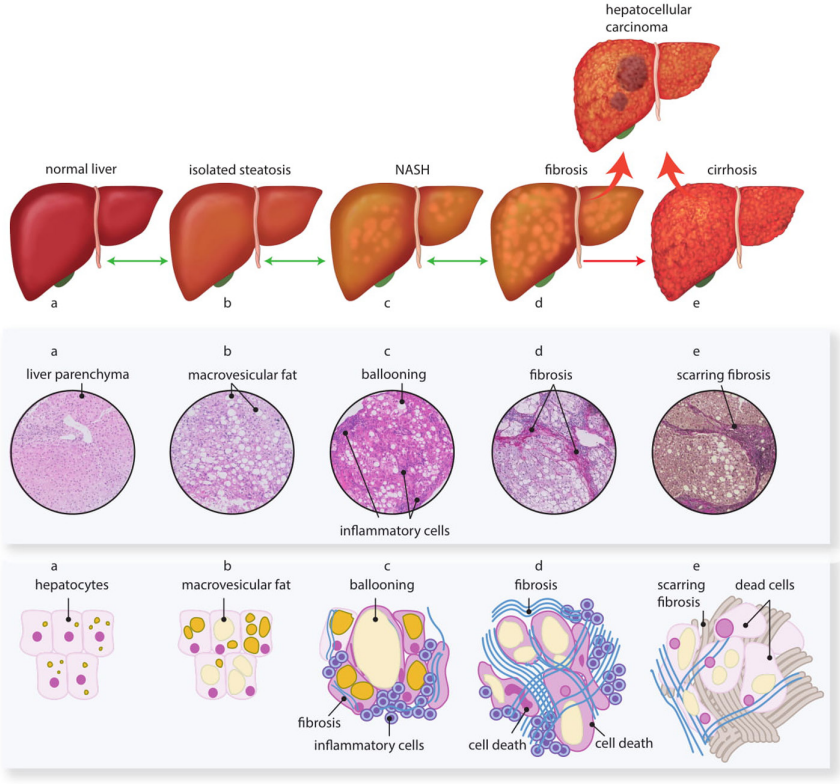


Figure 1
The disease spectrum of non-alcoholic fatty liver disease (NAFLD).

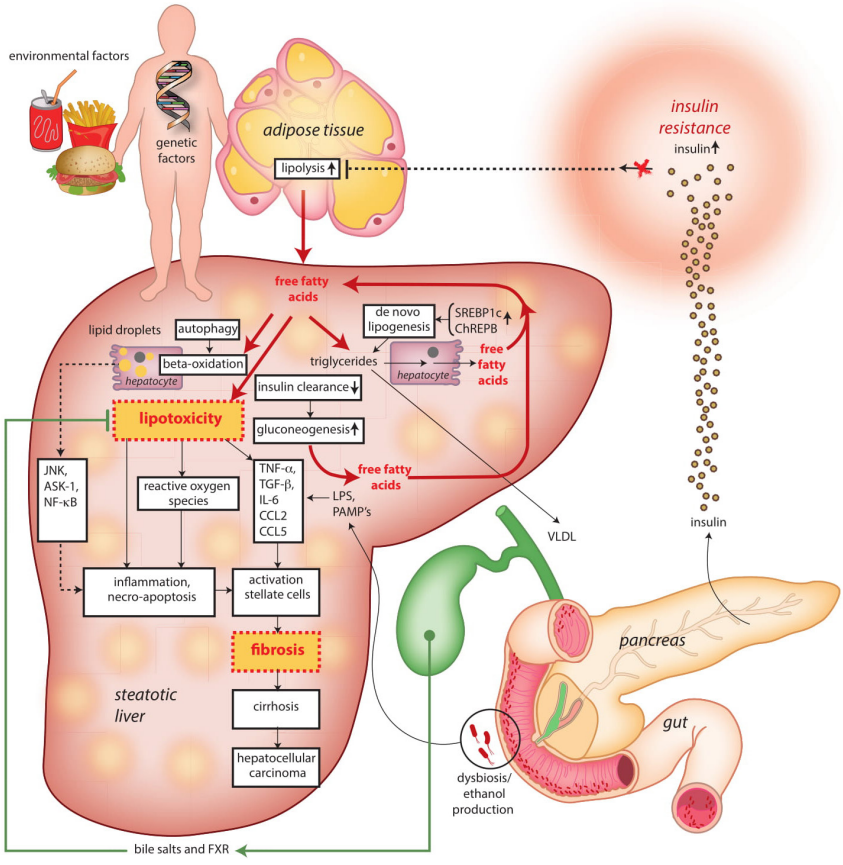


Figure 2
The pathogenesis of non-alcoholic fatty liver disease (NAFLD).

enhancing hepatic gluconeogenesis. As shown in Fig. 2, this cycle of processes causes an abundance of FFAs and triglycerides. When hepatic compensatory mechanisms fall short, lipotoxicity occurs, causing mitochondrial dysfunction, resulting in formation of reactive oxygen species (oxidative stress), inflammation and cell damage (15, 16, 17).

The overload of circulating FFAs triggers pro-inflammatory pathways (*c-jun terminal kinase* (JNK) via *apoptosis signal-regulating kinase 1* (ASK-1), and *nuclear factor kappa-light-chain-enhancer of activated B-cells* (NF-κB)), leading to inflammation, fibrosis and hepatocyte cell death or apoptosis (18, 19). Another pro-inflammatory pathway in NAFLD is formed by the production of cytokines by the visceral fat tissue, the so-called adipocytokines, such as IL-6 and TNF-α. These adipocytokines are directly transported to the liver through the portal system and cause a pro-inflammatory hepatic environment (18, 20, 21), resulting in activation of Kupffer and stellate cells and leading to fibrosis (22, 23, 24). Various other hepatokines have been shown to play a role in the development of NAFLD-NASH, as reviewed elsewhere (25).

Furthermore, dysregulation of the urea cycle may be involved in the pathogenesis of NAFLD, causing a build-up of toxic ammonia, potentially as a result of mitochondrial dysfunction (26). Many other factors have been implicated in the pathogenesis of NAFLD, including bile acid signalling and the gut microbiome (27, 28).

With respect to genetics, variations in genes involved in lipid metabolism and VLDL export (i.e. PNPLA3, TM6SF2, MBOAT7 and HSD17B13) have been found to exert an effect on the complex pathophysiologic mechanisms involved in the development of NAFLD and NASH (29, 30, 31, 32).

Diagnostics

There is a clear need for good diagnostic tests for NAFLD-NASH. The limited sensitivity of available tests (liver enzymes and ultrasound), as well as the limited implementation of more sensitive diagnostic modalities such as the Fibrosis-4 (FIB-4) score and vibration controlled transient elastography (VCTE), have turned this into a very active development area of the NAFLD research field, with a variety of diagnostic tests available and in development. The performance of diagnostic tests is linked to the prevalence within the tested population and

therefore, different tests should be used in primary care versus secondary or tertiary care and test results should be interpreted accordingly. Table 1 shows an overview of available tests, their contexts of use, costs, accuracy and (dis)advantages.

Most patients with NAFLD express (slightly) elevated serum liver enzymes, in particular ALT and γGT. However, liver enzymes within the reference range do not exclude NAFLD, and although elevated liver enzymes may serve as a diagnostic clue for the presence of liver disease, they fail to predict the presence and severity of hepatic steatosis, inflammation (NASH) and fibrosis (33). Therefore, various scores have been developed to estimate these aspects of NAFLD in a non-invasive way, such as the Fatty Liver Index (FLI) for steatosis (34) and the FIB-4 score for fibrosis (35).

FLI is an algorithm that combines BMI, waist circumference, γGT and triglyceride levels to predict the presence of hepatic steatosis with good accuracy as compared to MR or spectroscopy (36). However, it does not identify patients with more advanced disease (NASH or advanced fibrosis). Therefore, FLI is a useful tool in epidemiological studies, but it is not considered useful in a clinical setting (37).

The FIB-4 score is a calculative score based on age, AST and ALT levels and platelet count. It can be used to distinguish patients likely to have advanced fibrosis from those who do not, while keeping in mind the predictive value of the test for the specific patient at the chosen threshold (38). This test recently performed well in a care path in the United Kingdom and guides referral of patients to secondary health care for further analysis and surveillance for cirrhosis associated diseases including oesophageal varices and HCC.

The NAFLD fibrosis score (NFS) is a calculative score that takes into account an impaired fasting glucose or diabetes, age, AST and ALT, platelet count, BMI and albumin. This score performs similarly to FIB-4 for ruling out advanced fibrosis (39).

Another test aimed at identifying patients with liver fibrosis is the Enhanced Liver Fibrosis (ELF) test. This test combines three serum markers of hepatic matrix metabolism: hyaluronic acid, pro-collagen III amino terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase-1 (TIMP-1) and therefore more closely represents the biological process of fibrosis formation (40). It can also be used to monitor disease progression and response to treatment.

Thus far, no non-invasive biomarker or score exists that has both high sensitivity and specificity and is applicable to a wide population of patients and the search for such a marker is on-going. A plethora of biomarkers and scores are currently being tested and validated in large-scale European (LITMUS) (41) and American (NIMBLE) NAFLD biobank studies (35, 39, 42).

Ultrasonography, in which the reflection pattern of the liver is compared with the kidneys and/or spleen, is a tool often used to determine the presence and extent of hepatic steatosis (43). This diagnostic test is widely available (especially in primary care) and of low cost, although its sensitivity is limited in patients with moderate steatosis (<20%) and in those with a BMI > 40 kg/m^2 (44). Furthermore, ultrasonography cannot determine the presence and extent of inflammation (NASH) and fibrosis. Therefore, it can be used to rule out other causes of abnormal liver function tests, but it is not sufficient to be used for risk-stratification or to guide referral of patients suspected of NASH or fibrosis.

'Vibration controlled' transient elastography, VCTE™ or FibroScan® (brand name) is a non-invasive tool that can be used to measure the elasticity of the liver, thereby determining the presence and extent of fibrosis (45). This technique uses the simultaneous emission of both sonographic and electrical waves. Using the velocity of wave transmission through the tissue, it estimates liver elasticity: the faster the wave, the stiffer the tissue, as in fibrosis. By also using the extinguishment of the ultrasonography signal (the so called *continued attenuation parameter*, or CAP™), it estimates the amount of hepatic steatosis. VCTE/CAP was recently shown to be very accurate for both steatosis and cirrhosis over the incremental stages of NAFLD (45). Combined with its ease of use, we therefore expect the application of VCTE/CAP to increase in both primary and secondary care over the next few years, although the significant cost of the equipment may hamper this development.

The most accurate non-invasive method to diagnose and quantify hepatic steatosis is magnetic resonance imaging (MRI), especially using the so-called *proton density fat fraction* (PDFF) technique (46). Recently, a new multiparametric MR index has been proposed for diagnosing NASH, in which MR spectrography (MRS), MR elastography (MRE) and T1 mapping were combined in order to cover the various components of NASH. In a small group of 20 NASH and 27 non-NASH patients, this multiparametric MR index was shown to have an AUC of 0.883, which needs to be validated in larger NAFLD cohorts (47). However, because magnetic resonance imaging is time-consuming, expensive, of limited availability and its validity to measure fibrosis has not yet been confirmed, it is currently less suited for clinical use.

Table 1 Overview of the costs, contexts of use, accuracies, advantages and disadvantages of available diagnostic tools.

Diagnostic tool	Costs	Context of use	Accuracy				Advantages	Disadvantages
			Condition	Sensitivity	Specificity	PLR		
Serum liver enzymes (ALT, γGT)	<€5	Screening for potential liver disease	No available numbers on specificity and sensitivity of elevated serum liver enzymes for hepatic steatosis in adults				Widely available	No predictive value on presence and severity of hepatic steatosis, inflammation and fibrosis
			Accuracy of elevated liver enzymes for hepatic steatosis in children	44%	89%	4.00 (131)	Low costs	ALT low sensitivity for hepatic steatosis(132)
							Easy to use	γGT low specificity for liver disease(132)
							Non-invasive	
							ALT highly specific for liver disease (132)	
							γGT highly sensitive for hepatic steatosis (132)	
FIB-4 score (age, AST, ALT and platelets)	±€5	Screening for potential liver fibrosis in primary/ secondary care	For significant fibrosis (grade ≥3): (cut-off value 1.41)	71.9%	53.9%	1.56 (133)	Low costs	No information on the severity of fibrosis
							Easy to use	No predictive value for steatosis and inflammation
							Non-invasive	Limited specificity and accuracy
							Clinical relevance established in stepwise combination with ELF (UK) (49)	Not useful for follow-up
Enhanced Liver Fibrosis (ELF) (hyaluronic acid, PIIINP, and TIMP-1)	±€85(134)	Screening for potential liver fibrosis in primary/ secondary care	For significant fibrosis (grade ≥3)	83%	73%	4.00 (135)	Easy to use	No information on the severity of fibrosis
							Non-invasive	No predictive value for steatosis and inflammation

(Continued)

Table 1 Continued.

Diagnostic tool	Costs	Context of use	Accuracy				Advantages	Disadvantages
			Condition	Sensitivity	Specificity	PLR		
Abdominal ultrasonography	+/- € 125(136)	Diagnosing and evaluating extent of liver steatosis	For hepatic steatosis	84.8%	93.6%	13.25 (43)	Widely available	Limited sensitivity and negative predictive value(137)
							Low costs	Limited sensitivity in patients with moderate steatosis (<20%) and BMI > 40 kg/m^2
							Non-invasive	No information on presence and extent of inflammation and fibrosis
							High specificity for exclusion of steatosis >20%(137)	
Vibration controlled transient elastography (VCTE™ or FibroScan®)	+/- €220(138)	Determining presence and extent of liver fibrosis and steatosis	For hepatic steatosis	73%	69%	2.35(139)	Easy to use	Not available in every medical center
			For significant fibrosis (grade ≥3)		83.5%	5.05(140)	Non-invasive	Significant costs of equipment
							Accurate over all incremental stages of NAFLD(45)	
Proton Density Fat Fraction (PDFF-,)-Magnetic resonance imaging (MRI)	+/- €335(136)	Diagnosing and quantifying liver steatosis	For hepatic steatosis	96.6%	74.8%	3.83(141)	Non-invasive	Time consuming
							Most accurate diagnostic tool for liver steatosis	Significant costs
								Limited availability
								Non-validated diagnostic tool for fibrosis

Liver biopsy	+/- €1.250(138)	Diagnosing NASH and determining extent of liver fibrosis and steatosis	Gold standard	Accurate information on presence and severity of steatosis, fibrosis and inflammation	Risk of bleeding Risk of sampling error Inter- and intra-rater variability	Large inter-observer variability Invasive procedure

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGT, gamma-GT; NAFLD, non-alcoholic fatty liver disease; PIIINP, type III procollagen peptide, PLR, positive likelihood ratio; TIMP-1, TIMP metallopeptidase inhibitor 1; UK, United Kingdom

To date, liver biopsy remains the gold standard for diagnosing NASH, in which the characteristic swelling of hepatocytes (ballooning) and lobular inflammation can be established (Fig. 1). An important additional benefit of liver biopsy is the possibility to assess the presence and extent of liver fibrosis. However, liver biopsy is an invasive procedure that can be painful, has a risk of post-biopsy bleeding (up to 2%) and might convey a sampling error, due to only about 1/50.000th of the liver tissue being analysed, while NAFLD is often not equally distributed throughout the liver (48).

Clear numbers on specificity, sensitivity and potential risks of the diagnostic tools listed above are lacking because of large differences in methodology of clinical studies, which hampers their comparability. Therefore, diagnostic decisions should be made individually based on the specific case, the available diagnostic tools and the potential harms and benefits of the different diagnostic tools. Table 1 shows an overview of the advantages and disadvantages of available diagnostic tools (49).

Clinical consequences

Patients with NAFLD are at increased risk of atherosclerotic cardiovascular disease (asCVD). A recent meta-analysis showed that NAFLD was associated with an increased risk of fatal and non-fatal cardiovascular disease (odds ratio 2.58 (1.78–3.75)) (50). Interestingly, in patients with recently diagnosed myocardial infarction, the severity of stenosis on coronary angiography was found to correlate with the degree of hepatic steatosis on conventional ultrasound as well as VCTE-CAP (50). Moreover, the cumulative risk for mortality has shown to be increased in patients with coronary disease and stage 3-steatosis compared to patients with coronary disease and stage 1- and 2-steatosis (51, 52).

Whilst these studies all show a strong association between hepatic steatosis and cardiovascular disease, a recent European population-based cohort study with 120.795 NAFLD patients versus matched controls showed that when adjusting for age, smoking, diabetes, hypertension, total cholesterol levels and statin use the hazard ratios for acute myocardial infarction (AMI) are lower than firstly calculated (53). This suggests that NAFLD is likely not an independent risk factor for asCVD, but may be driving asCVD by exacerbating risk factors like hypertension, insulin resistance and dyslipidaemia (50, 54). Causal pathways by which NAFLD may drive asCVD are the mixed dyslipidaemia, as well as proinflammatory

and prothrombotic factors, most notably PAI-1. Favouring the lipid link and as mentioned previously, Mendelian randomization studies show that variation in the gene PNPLA3, involved in steatosis without affecting plasma lipid levels, does not increase the risk of asCVD, whereas variation in TM6SF2, affecting both steatosis and plasma VLDL, does increase asCVD risk (55). In addition to asCVD, NAFLD is related to cardiovascular disease by influencing left ventricular function. A recent study showed decreased left ventricular function within patients with NAFLD-fibrosis (56).

Given the strong association between NAFLD, mixed lipidaemia and asCVD, treatment with statins to lower the lipid levels and decrease the risk on the development of cardiovascular events is important and has shown to be safe in a variety of large studies (57).

Besides this increased risk of adverse cardiovascular outcomes, increasing evidence has shown NAFLD can develop into a seriously harmful disease also with regards to liver-related outcomes, contrary to previous beliefs (58). Especially in patients with active disease, i.e. NASH, the formation of fibrosis and cirrhosis increases morbidity and mortality. A recent meta-analysis has shown that for patients with NASH it takes circa 7.1 years to progress 1 stage in fibrosis compared to circa 14.3 years for patients with simple steatosis (59). Importantly, NAFLD patients with T2DM are at higher risk of developing advanced liver fibrosis than NAFLD patients without diabetes (60, 61). Data from the American Transplant Registry show that in 2018, NAFLD/NASH-related cirrhosis became the main indication for liver transplantation, overtaking other liver diseases such as viral hepatitis and alcoholic liver disease (ALD), and this indication is on the rise in Europe as well (62, 63). An additional problem is that the amount of liver donors with signs of NAFLD is also rising, increasing the risk of post-transplantation complications for the recipient (63).

Since liver cirrhosis is a well-known risk factor for development of HCC, patients with cirrhosis need to be regularly screened. However, not seldom HCC is diagnosed in patients that were unfamiliar to have NAFLD, even without cirrhosis (64). Possibly due to lack of screening, patients with NAFLD that develop HCC have decreased survival rates compared to HCC patients with underlying alcoholic liver cirrhosis (65). Screening for HCC in NAFLD patients is however debatable, since the incidence of HCC in NAFLD patients is low, demonstrated by a large meta-analysis that found an annual incidence of 0.44 per 1000 person-years. In patients diagnosed with NASH, the HCC incidence was 5.29 per 1000 person-years (5).

Treatment

The complexity of the various pathways involved in the development of NAFLD complicates the treatment of this disease. Given the strong association with obesity and T2DM, lifestyle changes and weight loss are major targets in treatment of NAFLD and NASH. However, the increasing knowledge on an abundance of pathophysiological mechanisms that are involved, combined with the increase in incidence and severity of this disease has led to an enormous pharmaceutical development (66, 67).

Lifestyle and nutritional interventions

Lifestyle intervention is the most important factor in the treatment of NAFLD (68, 69). A weight reduction of 8% has been shown to result in a 50% decrease in liver fat (69). Yet an increase in physical activity without any reduction in body weight also has a positive effect on hepatic steatosis (69). In patients with NASH, a strict 52-week programme of physical activity and diet resulted in a resolution of NASH in 25% of patients and 19% of patients showed regression of fibrosis. The extent of weight loss was associated with the level of improvement in histologic features of NASH. However, the vast majority of patients in this study did not reach the 5% weight loss goal, reflecting the difficulty of lifestyle changes in this specific population (70). On the other hand, studies have shown that doubling of daily calorie intake in healthy volunteers with so-called fast food resulted in elevated plasma ALT levels and increased steatosis within 4 weeks (71).

Especially foods and beverages containing large amounts of fructose, like sodas, have shown to have a cumulative effect on the development of NAFLD and even fibrosis (72). Lifestyle changes and weight reduction are expected to remain centrally important within the treatment of NAFLD, since they not only exert positive effects on NAFLD but also on associated metabolic and cardiovascular diseases. Even modest alcohol use has been shown to mitigate the ability of the body to resolve NASH and is associated with increased serum liver enzymes, suggesting patients with NAFLD should completely cease their alcohol use (73). Moreover, certain dietary changes, like the Mediterranean diet, consisting of mainly single unsaturated fats derived from fish or olive oil, help to reduce the amount of liver fat and insulin insensitivity, even without inducing body weight reduction (74). Limiting the amount of free sugars in the diet was also shown to have a positive effect on NAFLD (69).

Interestingly, the use of three or more cups of coffee a day protects against NAFLD and the formation of fibrosis, possibly through an increase in autophagia of lipid droplets within hepatocytes (75).

However, not only caloric intake, but also dietary composition has shown to play a role through dysbiosis of the gut microbiota, which most likely contributes to the development of NAFLD and NASH (27, 76, 77, 78, 79). A diet rich in lipids, animal-derived proteins and sugars provides a more favourable culture medium for certain bacterial species (like Bacteroides) than for others (80, 81). However, the mechanisms that underlie the development of NASH through microbiome imbalance are not fully understood. One hypothesis is that certain bacterial compositions increase gut permeability, thereby exposing the portal vein and liver to (products of) gut bacteria that induce various inflammatory pathways. Another hypothesis is that gut bacteria may induce or protect against NAFLD by producing pro- or anti-inflammatory metabolites. Both harmful metabolites, such as alcohol, as well as protective metabolites, like butyrate produced by *Eubacterium hallii*, have been identified as possible mechanisms in the development of hepatic steatosis in recent murine models (82). A recent study revealed that presence of high-alcohol-producing strains of *Klebsiella pneumoniae* in the gut was strongly associated with disease severity in a Chinese cohort of NAFLD patients, and that this strain could reproduce fatty liver disease in murine models. The endogenous alcohol production of these bacteria might activate similar molecular mechanisms as in fatty liver disease mediated by habitual excessive alcohol consumption, which is microscopically nearly indistinguishable from NAFLD (83).

Animal models suggest that influencing the gut microbial composition by using probiotics can reduce NAFLD (80). In humans, a recent placebo-controlled pilot study in 20 patients with biopsy-proven NAFLD revealed a decrease in hepatic steatosis after 6 months of treatment with probiotics consisting of various species such as *L. plantarum*, *L. delbueckii* spp. *bulgaricus*, *L. acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum*. A decrease in hepatic steatosis was found, which was associated with an increase in *Bacteroides* and a decrease of *Firmicutes* species (84).

Gut bacteria also play an important role in the modification of bile acids. The dysbiosis of the microbiome can lead to alterations in bile acid composition, potentially modifying the absorption and metabolism of lipids, resulting in a dysregulation of energy metabolism (28, 85).

Bariatric surgery and endobariatric procedures

Bariatric surgery has been proven to be very effective in NALFD patients, a reduction in body weight of over 10% can lead to complete regression of hepatic inflammation and fibrosis (86, 87). The Lille Bariatric cohort study showed that in morbidly obese patients with biopsy-proven NASH, bariatric surgery (i.e. gastric banding, sleeve gastrectomy and gastric bypass) resulted in a resolution of NASH in 85% of patients and a reduction in fibrosis (86). Gastric bypass has been shown to be more effective in improving NAFLD and NASH compared to other procedures (88).

Moreover, new endobariatric techniques have been developed to fill the gap for patients who do not qualify for bariatric surgery or prefer less invasive strategies. Endoscopic gastroplication has shown to be a durable, less invasive therapy providing results similar to sleeve gastrectomy. But also other endobariatric procedures like space-occupying devices, aspiration therapy and endoscopic small bowel bypass therapies have been proven to be successful in inducing weight loss (89, 90). A single-centre retrospective cohort study following 135 patients with obesity and NAFLD undergoing intragastric balloon (IGB) treatment revealed changes in BMI after 6 months, as well as corresponding improvement of ALT, GGT and insulin resistance (HOMA-IR) scores (91). However, as endobariatric interventions are often temporary or less durable than bariatric surgery, aggressive weight maintenance afterwards is key for lasting weight reduction.

Pharmacological developments

Blood glucose-lowering drugs

Due to the close link between NAFLD and glycaemic dysregulation, blood glucose-lowering drugs also retain therapeutic effects in patients with hepatic steatosis. Treatment with metformin, a mainstay therapy in T2DM, showed to be associated to mitigation of steatosis in patients with NASH (92) and to prevent hepatic events in T2DM patients with advanced NASH (93).

Whereas metformin mainly affects hepatic insulin resistance, GLP-1-receptor agonists and analogues (liraglutide and semaglutide, respectively exenatide) have direct effects on pancreatic insulin production and also impact the heart, brain and gastrointestinal system (94). Despite the lack of unambiguous data about a direct effect of these medications on the liver, studies have suggested liraglutide to have a positive effect on NASH and the

formation of fibrosis by reducing body weight, even in patients without T2DM (95). Moreover, liraglutide has shown to result in a reduction of asCVD events in patients with T2DM (96). However, strict rules for financial reimbursement for these medications, limiting off-label clinical use, and the need for s.c. injections to administer the drugs are hampering broader clinical implications. An oral form of the GLP-1 analogue semaglutide is currently in development for the treatment of NAFLD (66) and has already shown to have positive results in patients with T2DM in a phase 3-study (97).

Another group of therapeutics, SGLT2 inhibitors, such as canagliflozin, dapagliflozin and empaglifozin, selectively block the sodium glucose cotransporter 2 in the kidneys, thereby inhibiting renal glucose reabsorption in the proximal tubule. This increases urinary glucose excretion and reduces blood glucose levels in patients with T2DM. Results from multiple clinical NAFLD trials have shown SGLT2 inhibitors to significantly decrease serum liver enzyme levels compared to other oral glucose-lowering agents (98). Moreover, animal models of NASH demonstrated that treatment with SGLT2 inhibitors could prevent the development of hepatic steatosis and fibrosis, possibly by promoting fat utilization and by reducing de novo lipogenesis in the liver (99). In addition, canagliflozin has demonstrated to reduce asCVD events in patients with T2DM (100). However, SGLT2 inhibitors increase the risk of diabetic ketoacidosis, urinary tract infections and hypotension and dehydration, and the effect is minimized by the amount of serum glucose that can be excreted via the urine, which is estimated at 50%. The alleviation of hyperglycaemia and the induction of weight loss only partially explain the extent of NAFLD improvement with SGLT2 inhibitors, suggesting the involvement of other, still unknown pathways. Because of safety concerns and the limited effects of the established drugs on-going research is seeking novel/safer SGLT2 inhibitors.

Furthermore, thiazolidinediones like pioglitazone and rosiglitazone (withdrawn from the market in 2010), which increase insulin sensitivity, have been shown to exert positive effects on NAFLD through PPARγ-agonism (101, 102, 103). Unfortunately, they have disadvantages such as an increase in body weight, negative cardiovascular effects (mainly rosiglitazone) and a possible increased risk of bladder cancer (104).

Inflammation and necro-apoptosis

Selonsertib is an ASK1 inhibitor that was recently investigated in two phase 3-studies, one in F3-fibrosis and one in F4-fibrosis. Both studies report negative, that is, no significant effect of selonsertib on hepatic steatosis, inflammation or fibrosis (105, 106).

CCR2/5 antagonists

Cenicriviroc is a CCR2/5 antagonist, inhibiting macrophages in the peripheral fat tissue, which improves insulin sensitivity and inhibits migration, activation and proliferation of stellate cells. A phase 2 trial concluded cenicriviroc has positive effects on hepatic steatosis, inflammation and fibrosis and a phase 3-trial is currently being performed (107, 108).

Vitamin E

Vitamin E is a well-known antioxidant and exerts a positive effect on the amount of liver fat when prescribed in high doses (800 IE a day) (103). Unfortunately, no data have been collected on the effect of vitamin E on fibrosis, and high doses also seem to increase risk of prostate cancer and cerebrovascular accidents (CVA) (109, 110). The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines (developed in collaboration with the associations for diabetes and obesity) advise to consider vitamin E for patients with severe NAFLD (without T2DM) (3).

Lipid-lowering drugs

Although there have been safety concerns in the past about the prescription of statins in patients with elevated serum liver enzymes, these medications seem to have positive effects not only by decreasing risk of cardiovascular disease but also by inhibiting formation of hepatic fibrosis (111). Moreover, multiple agents are being developed to block the de novo lipogenesis within the liver, most notably ACC-inhibitors, and phase 2 and 3-trials are expected to read out in the near future (58).

Thyromimetics

Thyroid hormone and thyroid mimetics have the potential to reduce NAFLD-NASH. There are signs that these hormones exert positive effects on hepatic steatosis by mediating the induction of autophagia of lipid droplets and mitochondrial beta-oxidation of fatty acids (112). In 20 patients with T2DM and NAFLD, a low dose of thyroid hormone resulted in significant reduction of intrahepatic fat, measured by MRI-PDFF (113). A phase 2-trial in which

78 NASH patients were treated with a selective thyroid hormone receptor beta-agonist showed a significant reduction of intrahepatic fat measured by MRI-PDFF and an improvement of NASH on liver biopsy after 36 weeks (114). Therefore, a phase-3 trial with this selective thyroid hormone receptor beta-agonist is ongoing (115).

Bile acids

Besides their function in the absorption of lipids in the gut, there is accumulating evidence suggesting that bile acids also play a major role as signalling molecules in the liver and gut. Bile acids regulate the energy metabolism of lipids, sugars and proteins (28, 116). There are multiple on-going clinical trials using bile acid derivatives and mimetics as possible treatment for NASH (117, 118, 119, 120, 121, 122).

Ursodeoxycholic acid (a tertiary bile acid) and colesevelam (an anion exchange resin) have no effect on the amount of liver fat (65, 123). However, in the phase-2 FLINT trial the FXR-agonist obeticholic acid (OCA) did result in a histologic response of NASH in 46% of the participants with 25 mg a day (compared to 21% in the placebo group) (124). Unfortunately, a commonly reported side effect (23%) was pruritus, as well as an elevation in plasma LDL, due to increased lipoprotein lipase (LPL) activity. Interim analysis of the REGENERATE trial, a phase-3 trial concerning OCA in NASH, showed a small but significant reduction of fibrosis compared to the placebo group (125).

Combination therapy

Because of the complex pathogenesis of the disease, future medical treatment of progressive NAFLD is expected to consist of combination therapy, analogous to the current treatment of T2DM and hypertension. This expectation is so strong, that two types of combination therapy are already being investigated in clinical trials, even before the first monotherapy for NAFLD has been registered. In the phase-2 TANDEM trial, the FXR-agonist tropifexor is combined with cenicriviroc (126). In the phase-2 ATLAS trial, selonsertib is being combined with ACC-inhibition and a FXR-agonist. An early interim analysis did not show synergy in reducing hepatic steatosis on MRI-PDFF, yet the final results have to be awaited (127). Interestingly, both studies combine a drug that acts upstream (insulin sensitivity/lipotoxicity) in the pathogenesis of NAFLD with a drug that acts more downstream (apoptosis, fibrosis), which seems a highly plausible approach. Another recent randomized controlled trial investigated the combination of OCA and atorvastatin in NASH patients. It was shown that the OCA-dependent rise in LDL can be mitigated by atorvastatin, offering a potentially safer treatment option for NASH than OCA alone (128).

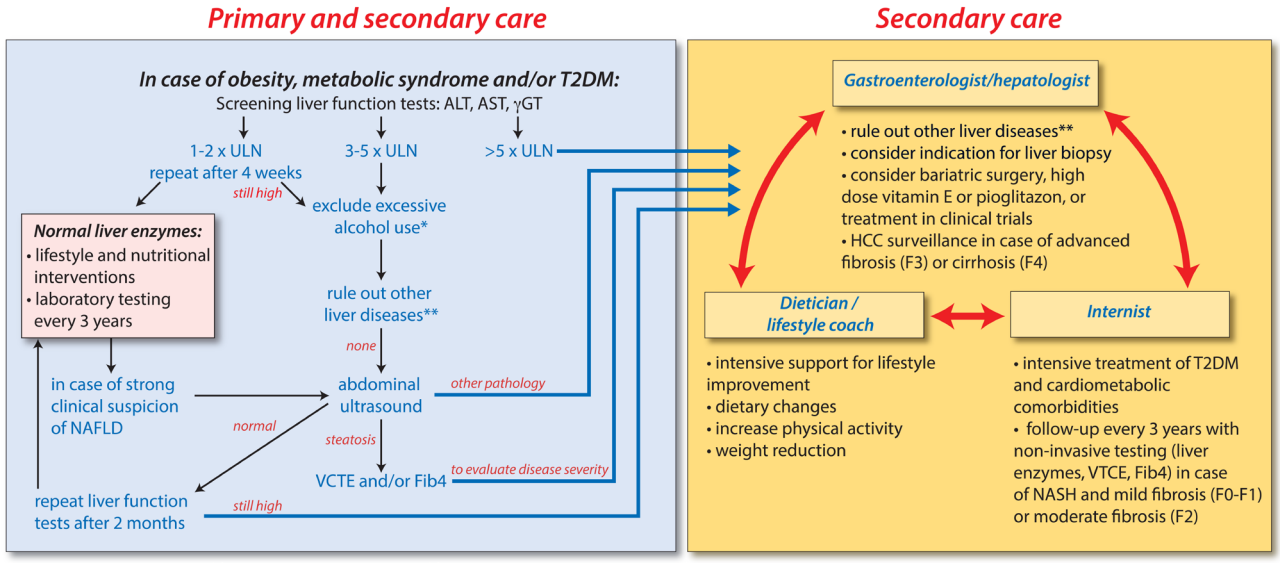
Multidisciplinary approach

NAFLD has become a serious health problem and patients with NAFLD often suffer from major cardiometabolic comorbidities. In recognizing patients with a high-risk profile for the development of NASH, the collaboration of the general practitioner, assistant nurse, internist-endocrinologist, vascular specialist and hepatologist is essential. In many medical centres across the globe, initiatives to develop a NASH-workgroup have sprouted, to stimulate this collaboration.

When patients are diagnosed with NAFLD, it is often not well recognized which patients suffer from non-progressive simple steatosis, which patients suffer from the metabolically active NASH with high risk of developing cardiovascular diseases and which patients have a high risk of developing cirrhosis and HCC. Developing more accurate and non-invasive diagnostic tools is necessary for a better capability of screening and differentiating between the different stages of liver disease, providing estimations for the chance of progression and the development of cirrhotic complications including HCC.

Meanwhile, in the absence of a widely available, accurate, non-invasive diagnostic tool, Fig. 3 provides recommendations for screening, diagnostics and surveillance in (suspected) NAFLD cases. It is advised to screen patients with a high risk for NAFLD by three-yearly measurements of serum liver enzymes or ultrasonography. In case of elevated serum liver enzymes or steatosis on ultrasound, screening for the presence of severe fibrosis or cirrhosis by VCTE™ or FibroScan® is indicated. In the presence of severe fibrosis or cirrhosis, surveillance of HCC should take place every 6 months. In case of portal hypertension screening for oesophageal varices is also indicated.

Another area of concern for these patients is the high risk of the development of cardiovascular diseases. Intensifying support for lifestyle improvement and treating comorbidities remains the cornerstone in halting the progression of liver disease and preventing cardiovascular complications of NAFLD. No clear pharmaceutical treatment has yet been established for NAFLD. However, considering the numerous on-going



* women: maximum 1 unit/day; men: maximum 2 units/day
** viral hepatitis, drug-induced liver diseases, auto-immune liver diseases, hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency

Figure 3
Multidisciplinary approach to non-alcoholic fatty liver disease (NAFLD).

trials, the development of therapeutic agents is expected in the near future. Until then, individual considerations have to be made in which therapeutic abilities, patient characteristics, patient preferences and potential harms and benefits of different treatment strategies should be taken into account. A multidisciplinary approach is essential to identify the patient population in need of this care and to ensure it being delivered. With this intent the flow chart in Fig. 3 has been composed, based on screening methods used in the United Kingdom and current guidelines (3, 35, 39, 45, 129, 130).

Conclusion

The increasing prevalence of NAFLD/NASH is worrisome, rendering this spectrum of liver disease a major global health problem. The challenge to distinguish mild stages from progressive stages of NAFLD and the upcoming advent of specific pharmacotherapy both require improvement of care paths for patients with NAFLD, guided by multidisciplinary guidelines and modules. Together this renders the clinical developments and scientific efforts within the field of NAFLD both very challenging and highly fascinating.

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Author contribution statement
M M R, A L M, U B, M E T and A G H wrote the manuscript together and interpreted the results. U B, M E T and A G H did the final review of the manuscript. M E T and A G H are the guarantors of this work and, as such, had access to all articles and take responsibility for the integrity of the article and the data mentioned. All authors approved the final version of the manuscript. M E Tushuizen and A G Holleboom are Joint senior author.

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