Management of non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease is a very common medical condition, driven by a combination of genetic and lifestyle factors, ultimately producing a severe chronic liver disease and increased cardiovascular risk. Most people are asymptomatic for a long time, and their daily life is unaffected, leading to difficulty in identifying and managing people who slowly progress to non-alcoholic steatohepatitis (NASH), NASH-cirrhosis, and eventually hepatocellular carcinoma. Despite advances in the understanding of pathogenic mechanisms and the identification of liver fibrosis as the strongest factor in predicting disease progression, no specific treatments have been approved by regulatory agencies. Outside controlled trials, treatment is generally limited to lifestyle intervention aimed at weight loss. Pioglitazone remains the drug of choice to reduce progression of fibrosis in people with diabetes, although it is often used off-label in the absence of diabetes. Vitamin E is mainly used in children and may be considered in adults without diabetes. Several drugs are under investigation according to the agreed targets of reduced NASH activity without worsening of fibrosis or improving fibrosis without worsening of NASH. Anti-inflammatory, anti-fibrotic agents and metabolism modulators have been tested in either phase III or phase IIb randomized controlled trials; a few failed, and others have produced marginally positive results, but only a few are being tested in extension studies. The development of non-invasive, easily repeatable surrogate biomarkers and/or imaging tools is crucial to facilitate clinical studies and limit liver biopsy.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a condition of excessive accumulation of fat in the liver of people consuming alcohol at amounts below risk levels.[1] The condition may be limited to excessive liver fat (NAFL) or progress to necroinflammation and fibrosis (non-alcoholic steatohepatitis (NASH)),[1] to NASH-cirrhosis,[2] and eventually to hepatocellular carcinoma.[3]

This definition carries two important biases: firstly, the necessary amount of liver fat remains undefined; secondly, no pathogenic insight exists, and diagnosis of NAFLD is excluded in people consuming alcohol above an uncertain and debated threshold. The safe limits of alcohol use, as set by European and American guidelines,[4 5] are limited to 20 g/day in females and 30 g/day in males. Importantly, the definition excludes even modest alcohol intake as a cofactor in accumulation of liver fat driven by the metabolic dysfunction. Several studies identified insulin resistance, with or without obesity, as the underlying mechanism associated with NAFLD,[6 7] and identified NAFLD as the hepatic expression of metabolic syndrome.[8]

To overcome the negative definition originally attributed to NAFLD, a proposal was put forward to change the term NAFLD to MAFLD (metabolic associated fatty liver disease),[9] assigning the disease a name linked with its pathogenesis. The new nomenclature is not yet accepted by regulatory agencies, and dissenting comments have been raised.

This review focuses on the latest evidence on screening methods to select patients for treatment and on therapies tested in randomized clinical trials (RCTs).

Sources and selection criteria

In PubMed, we retrieved 15 087 articles published between January 1980 and May 2020 by using the search term "non-alcoholic", "fatty liver", OR "steatosis" either [All Fields] OR [MeSH terms] AND "humans"[MeSH Terms], filtered by "Randomized controlled trial" and "Review". After prioritization of articles in English and exclusion of duplicate reports, the search included 778 randomized trials and 4099 review articles. We did further manual searching for additional articles on relevant databases (clinicaltrials.gov) and by scrutinizing

review articles for missing references. We added a few large observational studies for areas with few RCTs (for example, lifestyle intervention, most recent glucose lowering drugs) and excluded case reports and uncontrolled retrospective series.We included a few additional articles published up to 30 September 2020. Following revision, we included a few additional trials published up to 30 November 2020, to update the review with the most relevant clinical studies.

Epidemiology of NAFLD

The prevalence of NAFLD in the general population is about 25%, peaking at more than 30% in the Middle East and South America and as low as 13% in Africa.[10] Although NAFLD is associated with metabolic syndrome and obesity rates,[11] a recent meta-analysis of 84 studies (more than 10 million cases) concluded that 40.8% (95% confidence interval 36.6% to 45.1%) of patients with NAFLD were non-obese and 19.2% (15.9% to 23.0%) were definitely lean.[12] These rates were calculated with body mass index (BMI) adjusted for ethnicity—that is less than 23 for normal weight and 23.0-27.5 for overweight in Asians.

The prevalence depends on the method of ascertainment, specific clinical conditions (for example, obesity), and stage of disease. Ultrasonography is the reference technique for epidemiologic studies and in clinical settings but remains operator dependent and scarcely sensitive (only positive for liver fat ≥20-30% of the hepatic parenchyma).[13 14] More sensitive and quantitative methods have been developed for clinical trials, and surrogate biomarkers are used for epidemiologic studies. Using proton magnetic resonance spectroscopy (MRS),[15] the physiologic amount of liver triglycerides was set at 5.0%.[16] Surrogate non-invasive markers include unexplained elevated liver enzymes in patients with metabolic disturbances (namely, alanine aminotransferases) or specific algorithms (for example, Fatty Liver Index (FLI)).[17] According to the different techniques, the prevalence varies from a mere 3.2% (elevated aminotransferases, NHANES population)[18] to 19% (ultrasonography, same population)[19] and 34% (Dallas Heart Study population, proton MRS),[20] with age, sex, and ethnicity differences.[10]

The prevalence of NASH in the general population varies between 1.5% and 6.5% (that is, one in four to five patients with NAFLD),[10] but these estimates are derived from biopsy studies, with a high risk of selection bias. From a clinical point of view, the prevalence of advanced fibrosis, the key feature of progressive liver disease and liver related outcomes,[21] is measurable by non-invasive biomarkers[22] (preferably, the NAFLD Fibrosis Score (NFS),[23] Fibrosis-4 Index (FIB-4),[24] and Enhanced Liver Fibrosis (ELF) test[25]). The prevalence of advanced fibrosis (fibrosis, ≥F3)[26] in the general adult population is estimated at around 1.5%, and similar data have been obtained by non-invasive imaging methods (transient elastography (Fibroscan)).[27]

In obesity and type 2 diabetes, prevalence rates are increased twofold to fourfold,[28] depending on age and comorbidities. The prevalence of NAFLD in type 2 diabetes is estimated at above 60%,[29] with two thirds of biopsied patients having NASH and 10% having advanced fibrosis.[30-32] In obesity (BMI ≥30), the prevalence of NAFLD exceeds 60%,[33] and it exceeds 90% in morbid obesity.[34] Of particular concern is the prevalence of NAFLD among children (approximately 7.6% in the general population),[35] rising in parallel with obesity,[35] and the finding that overweight and obesity in childhood and young adulthood increases the risk of liver related morbidity and mortality in later life.[36]

Natural history of NAFLD

Progression of liver disease is extremely variable; pure fatty liver (NAFL) does not reduce life expectancy, whereas patients with NASH have increased all cause and liver related mortality.[37] Liver biopsy remains the sole method for a correct disease classification, but guidelines suggest limiting its use to very specific settings. The NAFLD activity score, calculated as the sum of steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2),[26] is largely used, but the European SAF (Steatosis, Activity, Fibrosis) score more precisely identifies the components of disease progression (fig 1).[38 39] Fibrosis is the most ominous predicting factor; it increases on average by one stage over 14.3 years in patients with NAFL and 7.1 years in patients with NASH.[40] In a recent meta-analysis of 4428 patients with biopsy proven NAFLD, the relative risks for events increased systematically from stage F2 onwards (significant fibrosis), to 3.42 (95% confidence interval 2.63 to 4.46) for all cause mortality, 11.13 (4.15 to 29.84) for liver related mortality, 5.42 (1.05 to 27.89) for liver transplant, and 12.78 (6.85 to 23.85) for liver related events in stage F4 (cirrhosis) compared with stage F0, irrespective of the presence of NASH.[21] In patients with F4 disease, liver decompensation occurs at rates of 3.3-15.6 per 100 person years, depending on Child-Pugh class.[41 42]

The whole cardiovascular system is often involved, driven by the atherogenic profile and features of metabolic syndrome.[43 44] Cardiovascular disease remains the most common cause of death[41]; diffuse atherogenic lesions, such as coronary artery disease and increased carotid intima-media thickness,[45 46] are more common in NAFLD, independent of traditional risk factors. Left ventricular failure and altered cardiac energy metabolism have also been described.[47]

NAFLD doubled the risk of incident type 2 diabetes in a meta-analysis incorporating data from 20 observational studies (nearly 117 000 people without diabetes), over a median five year follow-up.[48] The risk is diminished by resolution of NAFLD,[49 50] pointing to accumulation of liver fat as cofactor in the pathogenesis of type 2 diabetes.[51] Finally, the

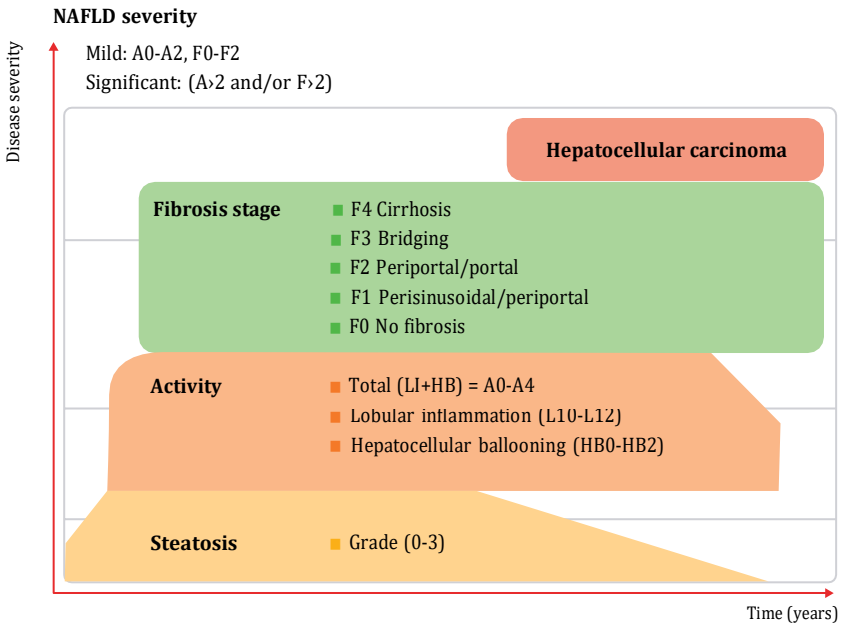

Fig 1 | Histologic classification of non-alcoholic fatty liver disease (NAFLD), according to European Steatosis, Activity, Fibrosis (SAF) score.[38] Non-alcoholic steatohepatitis is diagnosed by hepatocellular ballooning (HB) ≥1, independent of steatosis and lobular inflammation. Steatosis grade is not included in definition of disease severity. Note that steatosis may disappear in patients with advanced fibrosis (F3 and above); necroinflammation too tends to decrease, but less sharply than steatosis. Both steatosis and necroinflammation may fluctuate in response to intercurrent events

risk of incident chronic kidney disease is increased by 40% in association with type 2 diabetes.[52] Lean NAFLD, although characterized by an apparently lower severity (lower alanine aminotransferase concentrations, lower insulin resistance, and lower prevalence of features of metabolic syndrome),[53 54] shares a similar or even higher risk of disease progression.[53 55 56]

Hepatocellular carcinoma and extrahepatic cancers

NAFLD associated hepatocellular carcinoma is the third most common cause of hepatocellular carcinoma in the US (14%),[57] with a cumulative incidence of 2.4-12.8% over a median follow-up of 3.2-7.2 years.[58] NAFLD patients with advanced fibrosis (F3-F4) have an almost sevenfold increased risk of hepatocellular carcinoma compared with controls,[57] and the risk can be even higher in type 2 diabetes and obesity.[59] At diagnosis, patients with NAFLD related hepatocellular carcinoma are older and have a higher prevalence of extrahepatic comorbidities compared with viral or alcohol related hepatocellular carcinoma but a lower prevalence of cirrhosis (only two thirds of cases),[58] leading to less systematic surveillance and late diagnosis.[60] Accordingly, patients with NAFLD related hepatocellular carcinoma may receive less treatment and be more likely to die of their cancer,[61] despite a lower prevalence of cirrhosis leading to higher resection rates (19% *v* 11% in hepatitis C virus related hepatocellular carcinoma).[62]

All cancer related mortality is also increased, occurring in 1-2% of cases, possibly driven by metabolic alterations.[62] A large community cohort study showed that NAFLD was associated with a nearly twofold risk of extrahepatic cancers (particularly of the uterus, stomach, pancreas, and colon) during a median follow-up of eight years.[63] The association with incident cancer risk is stronger in NAFLD than in obesity,[63] suggesting that NAFLD might be the link between obesity and cancer.[64]

Screening

The natural history of NAFLD underlines the importance of timely diagnosis to reduce the burden of disease and the direct and indirect costs. Effective screening in the community and in selected cohorts is mandatory to define treatment strategies, but not all screening criteria are fulfilled for NAFLD.[65] In particular, we still lack an easy to repeat, cheap, and community acceptable test to assess disease severity, and treatment is limited to lifestyle intervention. Guidelines from the European Association for the Study of the Liver (EASL) suggested universal screening for NAFLD in patients with metabolic diseases,[5] according to resource availability. This position was criticized,[66 67] although limited to patients at higher risk of disease progression, and, as of 2019, the US guidelines do not support screening.[4] Universal screening is not cost effective,[68] but the cost-utility of screening procedures to select patients for biopsy, follow-up, and treatment is high, particularly in younger patients (below 45 years),[69 70] and programs for referral of patients with advanced disease to diagnostic procedures are needed. Two strategies are supported by all guidelines, with differences in relation to setting. The first is community screening, ideally by primary care physicians, using cheap, non-

invasive surrogate markers of steatosis and fibrosis (listed in the supplementary table), in particular FLI, FIB-4, NFS, and ELF test.[17 23-25] The second is screening by non-invasive markers, also including transient elastography,[71 72] by diabetes specialists in patients at higher risk of disease progression. In both cases, patients identified as having advanced disease should by referred to hepatologists for definite diagnosis (including liver biopsy), appropriate follow-up, and treatment. Biopsy is mandatory for patients entering clinical trials, as well as in case of conflicting results or competing diagnoses (table 1).

Primary care physicians are at the forefront in the community for early selection of people at risk. A two step screening procedure by FIB-4 index and ELF test (tools having a high negative predictive value) reduced unnecessary referrals to liver specialists by 81% and increased the referral of cases with advanced fibrosis by fivefold versus standard care.[76] This strategy also increased the detection of cases with cirrhosis in the community. Transient elastography as a second step or as the sole diagnostic procedure was similarly cost effective.[77] Effectiveness is likely to increase further in selected cohorts at higher risk of progression to hepatocellular carcinoma, such as diabetes cohorts. However, awareness of NAFLD among primary care physicians and non-liver specialists remains low,[78 79] and this extends to patients.

Pathophysiologic approach to treatment

Whereas simple steatosis is a reflection of non-progressive dysfunctional metabolism, NASH is a chronic liver disease that may progress undiagnosed for years, eventually emerging with liver failure and hepatocellular carcinoma. The burning question is why in some people a metabolic disease will translate into a progressive liver disease. NASH stems from a combination of environmental and genetic factors (fig 2); however, it is a network of interacting factors that drive the development NASH. Unraveling these factors is essential for risk stratification and provides a roadmap of potential therapeutic targets.

Lipotoxicity

The earliest events initiating NAFLD reside in an absolute or relative calorie excess, as confirmed by the link between NAFLD and obesity. Limited physical activity, sedentary behaviors, and screen watching are complementary aspects of calorie imbalance, irrespective of BMI.[80-84] Increased substrate flux will overload adipose tissue compartments, leading to dysfunctional adipose tissue, spillover of free fatty acids into non-adipose tissues, de novo lipogenesis, and accumulation of lipids in the liver. This process has been described by Unger as "lipotoxicity"[85] and occurs primarily in the liver (NAFLD), in the pancreas (non-alcoholic fatty pancreas, favoring type 2 diabetes), in the heart, and diffusely in the arterial circulation (atherosclerotic cardiovascular disease).

Under such circumstances, the liver, adipose tissue, muscle, and gut interact via cytokine, growth factor, and adipokine secretion, with the liver taking center stage in metabolic regulation. These multiple insults synergistically drive the development and

Table 1 | Comparative analysis of different guidelines on non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH)

Recommendation	EASL-EASD-EASO[5]	AASLD[4]	NICE[73]	Asian-Pacific[74 75]
Diagnosis (after excluding alcohol and secondary causes)	Steatosis by imaging or histology or unexpectedly high liver enzymes	Steatosis by imaging or histology	Any evidence of excessive liver fat, regardless of liver enzymes. Use Fatty Liver Index if testing adults for NAFLD	Steatosis by ultrasonography or transient elastography as first step (where available)
Community screening	Not cost effective	Not considered	Non-effective	Cost effectiveness unknown
Screening in high risk patients	All patients with one or more features of metabolic syndrome	Not mentioned	Not mentioned. Consider that NAFLD is common in type 2 diabetes and metabolic syndrome	Consider in patients with type 2 diabetes and obesity
Screening by non-invasive tests	NFS or FIB-4, followed by elastography	NFS, FIB-4, and elastography	ELF test	Biomarkers and imaging effective (no specific test)
Genetic screening	Not cost effective	Not mentioned	Not mentioned	Cost effectiveness unknown
Screening for complications	Define cardiovascular and diabetes risk	Define cardiovascular and diabetes risk	Define cardiovascular and diabetes risk	Define presence of all features of metabolic syndrome
Follow-up	Not at risk of progression, every 2 years; at risk, every 6 months	Not defined	Every 3 years in patients not at risk of progression; if at risk, use NICE guidelines for cirrhosis	Not mentioned
Liver biopsy	Mandatory in drug trials	Consider in patients at risk for NASH or advanced fibrosis and/or to exclude other coexisting liver disease	Gold standard, but not feasible also in patients at risk	When the diagnosis is unclear or when fibrosis assessment by non-invasive tests is inconclusive
Treatment: diet and weight loss	Dietary restriction (deficit 500-1000 kcal/day). Prefer Mediterranean diet	Dietary restriction (deficit 500-1000 kcal/day). No specific diet	Consider NICE guidelines for obesity and weight gain prevention. No specific diet	Consider multidisciplinary approach. Dietary restriction (deficit 500-1000 kcal/day)
Treatment: physical activity	Aerobic or exercise training (150-300 min/week), 3-5 sessions	Aerobic or exercise training (>150 min/week)	Consider NICE guidelines for obesity and weight gain prevention	Aerobic or resistance exercise (moderate intensity ≥150 min/week or vigorous intensity ≥75)
Treatment: drugs	Pioglitazone (off-label in absence of diabetes). Vitamin E not indicated. Other drugs not indicated	Pioglitazone and vitamin E in patients with/without diabetes, respectively. Other drugs not indicated	Consider pioglitazone in diabetic and vitamin E in non-diabetic cases with advanced fibrosis (only in secondary or tertiary care settings)	Consider pioglitazone for short term use in diabetes or prediabetes. Consider vitamin E in non-cirrhotic, non-diabetic NASH. Other drugs not indicated

AASLD=American Association for the Study of Liver Diseases; EASL=European Association for the Study of the Liver/European Association for the Study of Diabetes/European Association for the Study of Obesity; ELF=Enhanced Liver Fibrosis; FIB-4=Fibrosis-4 index; NFS=NAFLD Fibrosis Score; NICE=National Institute for Health and Care Excellence.

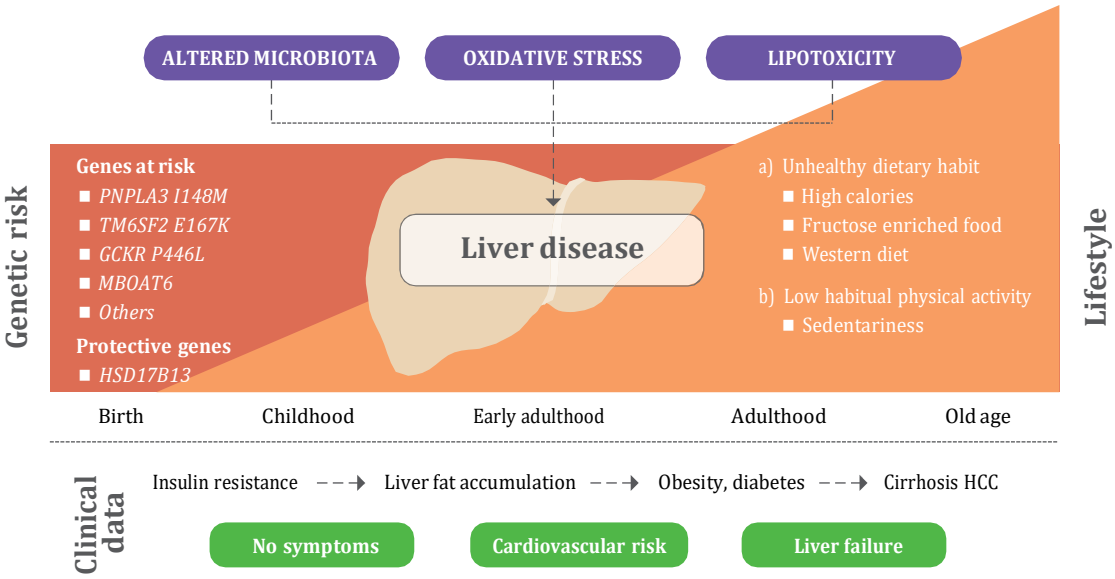

Fig 2 | Pathogenesis and progression of non-alcoholic fatty liver disease. Disease may proceed asymptomatically to cirrhosis or liver failure, sometimes heralded by events associated with cardiovascular risk. HCC=hepatocellular carcinoma

progression of NAFLD, particularly in genetically predisposed people.[86] NASH is much less prevalent than simple steatosis in the general population and does not correlate with severity of steatosis.[87] This suggests that most people with fatty liver are able to compensate for stressors that drive the progression to NASH in others. Triglycerides are not in themselves hepatotoxic, and hepatocyte injury is likely generated by toxic precursors or products of triglyceride metabolism. Besides free fatty acids, candidate lipotoxic lipids include monoglycerides and diglycerides, ceramides, dihydroceramides, and lysophosphatidyl choline species, as well as hepatic cholesterol accumulation, which may be responsible for necroinflammation,[88] [89] whereas other lipids (monounsaturated and polyunsaturated fatty acids) may exert a protective effect.[90]

Increased de novo lipogenesis from carbohydrates, specifically fructose,[91] [92] is expected to produce similar lipotoxic effects; consumption of sugar sweetened drinks containing either fructose or sucrose (converted to fructose and glucose in the gut) may be even more toxic than lipids in promoting NASH.[93] Uncontrolled and incomplete lipid oxidation, oxidative stress, and activation of the unfolded protein response are two well characterized pathways that promote cell death in NASH.

Gut microbiota

An altered microbiome ("dysbiosis") may contribute to liver damage. Human studies document a fecal microbiome signature characterized by increased Proteobacteria and Bacteroidetes along with a decrease in Firmicutes in patients with obesity and NASH.[94] Mechanistic links between altered microbiome and NASH include increased intestinal permeability and bacteria modulation of the gut-liver axis through intestinal farnesoid X receptor (FXR) signaling, which regulates the transcription of genes involved in bile acid synthesis and transport, lipogenesis, and glucose homeostasis, either directly or indirectly, via release of fibroblast growth factor-19 (FGF19).

Gene polymorphisms

Ethnic differences in hepatic fat accumulation have long been described and lead to higher disease prevalence in people of Hispanic and Asian origin and lower prevalence in Africans and African-Americans.[95] Genetic differences are in keeping with twin and family studies showing that steatosis and progression of NAFLD to fibrosis and eventually to cirrhosis may be strong heritable traits.[96-98] Since the original finding of a close relation of liver fat with a polymorphism in the patatin-like phospholipase domain-containing 3 gene *(PNPLA3)*,[99] other genes accounting for an increased susceptibility to NAFLD have been identified by genome-wide association studies (table 2).[100] They act through totally different mechanisms, interacting with dietary factors, physical activity, and comorbidities, sometimes producing epigenetic effects.[101-105] Of note, they are also differently associated with cardiovascular disease, potentially driving outcome. A novel gene variant reducing the risk of liver disease has also been described (a loss of function variant of hydroxysteroid 17-β dehydrogenase 13 gene, *HSD17B13*),[106] as well as other polymorphisms linked with specific proteins in selected cohorts, offering a rationale for treatments.[101]

Fibrogenic response

Progression to liver fibrosis reflects the convergent impact of environment, metabolism, microbiome,

genetic risk factors, and comorbidities on cell death. In turn, dying hepatocytes trigger regenerative responses, enriching the liver with regenerative cells (myofibroblasts, immune cells, and liver cell progenitors).[107] Liver fibrosis is the result of repeated and protracted wound healing, ultimately driven by hepatic stellate cells, and reflects the net balance between fibrogenesis and fibrosis degradation. In NASH, ongoing fibrogenesis does not proceed linearly from simple fatty liver through NASH to cirrhosis. Rather, progression seems to result from repetitive necroinflammatory bouts interrupted by anti-inflammatory, reparative immune responses. Over time, futile regenerative responses also perpetuate the stimulus for neoplasia, increasing the risk of liver cancer.

According to the above mechanisms, treatment targets include attempts to repristinate calorie balance and lipid and glucose homeostasis, to reduce oxidative stress and systemic and local (hepatic) inflammatory signals, or to modulate stellate cell activation and fibrogenesis. Pleiotropic drugs such as FXR agonists and glucagon-like peptide-1 (GLP-1) receptor agonists hit more than one target within the injury milieu. As both the mechanisms leading to NASH and their phenotypic expression are highly heterogeneous, treatment should theoretically be tailored to individual patients and potentially consider combination therapy.

Treatment

Lifestyle intervention

Lifestyle intervention is the fundamental and, currently, the sole treatment of NAFLD, as long as no drugs are approved by regulatory agencies. The favorable effects of weight loss on surrogate biomarkers and imaging tests have been extensively demonstrated in observational studies, but only a few RCTs are available and very few are based on histological outcomes. An exhaustive analysis of this evidence is outside the scope of this article, and several comprehensive reviews are available.[108-111]

Calorie restriction and physical activity are consistently recommended in guidelines (table 1). Both aerobic and resistance exercise are recommended, and usually no specific diets are suggested, with a general indication to reduce intake of simple sugars, industrial fructose, and saturated fats, and with a preference for the Mediterranean diet in the European recommendations.[5] The most relevant observational studies and a few recent RCTs are discussed below, with details provided in table 3.[112-119]

The first robust evidence for the beneficial effects of intensive lifestyle intervention (ILI) programs on NAFLD came from studies conducted using the strategy of the Diabetes Prevention Program,[120] based on cognitive-behavioral treatment carried out by a dedicated team. In patients with or without type 2 diabetes,[112 113] ILI significantly reduced body weight and intrahepatic fat, assessed by MRS,[121] and improved liver histology.[113] Of note, beneficial effects were also observed in control participants achieving pre-defined weight loss targets (weight loss ≥7% of initial body weight).[113] The results were confirmed in a much larger sample of patients with ultrasonographically detected NAFLD, in which ILI was also associated with improved metabolic and cardiovascular risk factors.[114] In a community based study, patients treated with ILI had a higher probability of remission of NAFLD and reduced fibrosis (MRS and transient elastography) compared with standard care.[115] In the same population, a 7-10% weight loss was later confirmed to achieve clearance of liver fat in NAFLD with obesity, whereas a 3-5% loss was similarly effective in lean NAFLD (BMI <25),[122] underlining the universal importance of diet and exercise to reduce prevalence and progression of NAFLD, and also improving health related quality of life.[123]

Table 2 | Genes involved in non-alcoholic fatty liver disease (NAFLD) and in progression of NAFLD

Gene	Metabolic effects	Prevalence in NAFLD and clinical significance
Patatin-like phospholipase domain-containing 3 (*PNPLA3 I148M* variant: adiponutrin)	Mutated protein accumulates on surface of lipid droplets preventing export from hepatocytes and favoring inflammation in hepatic stellate cells by interaction with retinol	10% *v* 5% in people of European ancestry (10-15% in Asian populations); 16% in NASH, 35% in NASH-cirrhosis, and 45% in NASH-HCC
		To be considered as possible marker of disease progression
Transmembrane 6 superfamily member 2 (*TM6SF2 E167K* variant)	Decreased lipid secretion in VLDL, leading to reduced circulating lipids (both cholesterol and triglycerides)	13% *v* 7.2% in people of European ancestry, 3.4% in African-Americans, and 4.7% in Hispanic-Americans
		Increased risk of NASH and advanced fibrosis
		Reduced risk of cardiovascular disease (hazard ratio 0.67), totally explained by low cholesterol concentrations
Membrane bound O-acyltransferase domain-containing 7 (MBOAT7)	Variant promotes changes in hepatic phosphatidylinositol acyl-chain remodeling	Increased risk of NAFLD along whole disease spectrum
		Predisposes to cirrhosis in alcohol misusers
Glucokinase regulator (*GCKR P446L* variant)	Variant impairs glucokinase inhibition in response to fructose-6-phosphate, thus blocking fatty acid oxidation	Associated with steatosis in children and adults and with presence of obesity, irrespective of ethnicity
		In NAFLD, predicts risk of fibrosis (≥F1)
Hydroxysteroid 17-β dehydrogenase 13 (HSD17B13)	Truncated protein has reduced enzymatic activity	Loss of function variant of gene protects against chronic liver disease (both alcoholic and non-alcoholic) and reduces risk of progressive NASH
		Reduces negative effects of *PNPLA3* variant

Genome-wide screening for genes in patients at risk of NAFLD and NAFLD progression is not currently advised by international and national guidelines.
HCC=hepatocellular carcinoma; NASH=non-alcoholic steatohepatitis; VLDL=very low density lipoprotein.

Table 3 | Principal lifestyle intervention studies for treatment of non-alcoholic fatty liver disease (NAFLD)

Author, year	Type of study; No of patients	Treatment and duration	Study target and outcome measures	Results
Lazo et al, 2010[112]	RCT; 96 T2DM	Intensive LS intervention (ILI, n=46) *v* diabetes support and education (DSE, n=50); 12 months	7-10% WL. Biochemistry; intra-abdominal fat (steatosis ≤5.5% IHTG at MRS)	Data collected as part of LookAhead study. At 1 year, ILI participants lost more weight (WL −8.0% *v* −0.5%) and had larger decline in IHTG content (−50.8% *v* −22.8%) *v* participants in DSE
Promrat et al, 2010[113]	RCT; 31 biopsy proven NASH	Intensive LS intervention (ILI, n=21) *v* standard care (SC, n=10); 48 weeks	WL ≥7%, improved biochemistry; reduced NAS (≥3 points) or post-treatment NAS ≤2; NASH remission at histology	WL 9.3% (SD 7.5) in ILI *v* 0.2% (6.1) in SC; NAS target reached in 72% *v* 30% (SC). In patients who achieved ≥7% WL, liver fat, ballooning, and lobular inflammation were improved, irrespective of treatment arm. Percent WL correlated with reduced ALT, steatosis, and activity
Sun et al, 2012[114]	RCT; 1087 NAFLD (ultrasonography)	LS treated (LS, n=724) *v* basic education (SC, n=363); 12 months	WL and liver enzymes; energy intake ≤25-30 kcal/kg BW; PA ≥23 METs/h/week + 4 METs of exercise. Visceral fat area by CT	WL larger in LS (−11.6% *v* 0.4% in SC); liver enzymes, IR, and parameters of MetS showed a larger improvement in LS *v* SC at 6 and 12 months. VFA was reduced in LS at 12 months
Wong et al, 2013[115]	RCT; 154 NAFLD (IHTG ≥5% and high ALT)	Intensive LS intervention (ILI, n=77) *v* standard care (SC, n=77); 12 months	NAFLD remission (IHTG content <5%), WL, changes in ALT, improvement in fibrosis (transient elastography)	ILI was associated with NAFLD remission (64% *v* 20% SC; difference 44%, 95% CI 30% to 58%), normal ALT (53%), and reduced fibrosis. 39% of ILI patients and no patient in SC had WL ≥10% (difference 39%, 28% to 50%). 97% of cases who achieved 10% WL target had NAFLD remission
Vilar-Gomez et al, 2015[116]	Cohort study; 293 biopsy proven NASH	All treated by intensive LS intervention (ILI); 261 cases had follow-up biopsies; 52 weeks	NASH resolution without fibrosis worsening; NAS improvement (≥2 points); improved histological lesions (≥1 point)	WL was ≥5% in 30% of cases. NASH remission was observed in 25%, NAS reduction in 47%, and fibrosis regression in 19%. Amount of WL was independently associated with improvement in all histological parameters (ORs 1.1-2.0). WL ≥10% was associated with NASH remission (90% of cases) and fibrosis regression (45%)
Khoo et al, 2017[117 118]	Pilot RCT; 24 obese MRI diagnosed NAFLD	Liraglutide (3 mg/day, n=12) *v* LS (diet and exercise, n=12); 26 weeks + 26 weeks of weight loss maintenance	WL, biochemistry, MRS elastography	Similar reduction in BW (−3.5 kg in both arms), liver enzymes, and liver stiffness (LS −0.21 kPa; liraglutide −0.26); liraglutide as effective as structured LS modification. at 52 weeks; liraglutide group significantly regained weight (+1.8 (SD 2.1) kg) and IHTG content (4.0% (5.3)), which were unchanged in LS group
Mazzotti, 2018[119]	Observational, cohort study; 716 ultrasonography assessed NAFLD	Web based LS program (WEB, n=278) *v* group based intervention (GROUP, n=438); follow-up, 2 years	WL ≥10%, changes in liver enzymes, surrogate markers of steatosis and fibrosis (FLI, NFS, Fib-4)	Attrition rate was higher in WEB (OR 1.87, 95% CI 1.20 to 2.90, at 6 months and 2.95, 2.04 to 4.26, at 2 years). 10% WL target was reached in 20% (WEB) *v* 15% (GROUP). 10% WL after 2 years was associated only with baseline BMI (OR 1.43, 1.13 to 1.81, per BMI/5). After adjustment for confounders and attrition, probability of reaching long term 10% WL was not reduced in WEB (OR 0.70, 0.38 to 1.27) *v* GROUP care

ALT=alanine aminotransferase; BMI=body mass index; BW=body weight; CT=computed tomography; Fib-4=Fibrosis-4 index; FLI=Fatty Liver Index; IHTG=intra-hepatic triglyceride; IR=insulin resistance; LS=lifestyle; MetS=metabolic syndrome; MRS=magnetic resonance spectroscopy; MRI=magnetic resonance imaging; NAS=NAFLD activity score; NFS=NAFLD fibrosis score; NS=not significant; OR=odds ratio; PA=physical activity; RCT=randomized controlled trial; SC=standard care; T2DM=type 2 diabetes mellitus; VFA=visceral fat area; WL=weight loss.

A Cuban observational study in 2015 provided a landmark step in support of the effectiveness of ILI in NAFLD, with its large sample size and number of histological assessments (293 cases, 261 follow-up biopsies).[116] The study confirmed a dose-response relation between weight loss at 12 months and remission of NASH and set 10% weight loss as the target for regression of fibrosis. Unfortunately, no data on long term follow-up have been published, nor on maintenance of weight loss, the critical factor in behavioral treatment.

ILI requires a dedicated team, rarely present in liver units, and continuing patient-therapist interaction, limiting participation and adherence and increasing costs. These limits may be partly overcome by e-technology; in 278 motivated, young NAFLD patients, weight loss targets, dietary adherence, and physical activity could be similarly achieved and maintained at two year follow-up by a web based program, compared with a group based educational approach, after adjustment for baseline differences.[119] The opportunities offered by new technologies for continuing motivation, support, and education toward lifestyle changes need to be exploited, as they can reach larger groups of at risk patients.

Finally, very few studies directly compared ILI and drug treatment in patients with NAFLD, using drugs approved for obesity or type 2 diabetes. A 26 week RCT did not show any difference between liraglutide (3 mg/day) and ILI on weight loss, biochemistry, and measures of fibrosis.[117] However, ILI was associated with sustained weight loss and reduced liver fat at follow-up, whereas weight regain and re-accumulation of hepatic fat occurred after liraglutide was stopped.[118]

Bariatric surgery

Bariatric surgery very effectively promotes weight loss and its maintenance; the effects on body weight largely exceed the 10% weight loss target associated with clearance of liver fat, resolution of NASH, and reversal of fibrosis. Accordingly, surgery is a possible treatment to reduce the burden of NASH in patients who meet the agreed criteria for the management of obesity (BMI ≥40 or BMI ≥35 with comorbidities). Roux-en-Y gastric bypass and sleeve gastrectomy are the procedures of choice,[34 124] and surgical treatment becomes cost effective in patients at high risk of progression (F3 fibrosis).[125]

The evidence supporting bariatric surgery is exclusively derived from observational studies, in which liver histology was measured at surgery and follow-up.[126] In 1236 cases, improvement of NAFLD, including regression of fibrosis, was associated

with five year post-surgery weight loss.[124] Notably, persistence of NASH one year after surgery was associated with less weight change (BMI −9.1 (SD 1.5)) than NASH resolution (−12.3 (0.6)).[34] In a retrospective analysis of a large insurance database, NAFLD patients with obesity who had bariatric surgery were less likely to progress to cirrhosis than matched cases not receiving surgery (hazard ratio 0.31, 95% confidence interval 0.19 to 0.52).[121] In a French bariatric cohort prospectively undergoing repeated biopsies, NASH had resolved at five years, without worsening of fibrosis, in 54/64 (84%, 95% confidence interval 73% to 92%) patients, and fibrosis decreased progressively in 70% and completely disappeared in 56% (42% to 69%) of cases, including 46% of patients with bridging fibrosis at baseline.[127] Cirrhosis in itself is not a contraindication to bariatric surgery, but a precise evaluation of hepatic functional reserve, portal hypertension, and cardiovascular risk factors is needed.[128]

Very recently, bariatric/metabolic endoscopy has been proposed to facilitate rapid and large weight loss, particularly in type 2 diabetes. These procedures include endoscopic sleeve gastroplasty, endoscopic small bowel bypass, and duodenal mucosal resurfacing. Although apparently safe and effective in the short term,[129] [130] much more data on histological outcomes and adverse events are needed for their extensive clinical application.

Drug treatment

On the basis of evidence from longitudinal studies, patients with intermediate and advanced fibrosis (F2-F4 fibrosis, usually described as significant fibrosis) are at greatest risk of overall and disease specific mortality and have been identified as the target population for investigational drugs in phase II-III trials. As patients who are in pre-cirrhotic stages are not at short term risk for liver related outcomes, regulatory authorities accepted histological features as surrogates of liver related events for accelerated or conditional approval, with the requirement that additional studies are done to determine whether short term changes translate into reduced progression to cirrhosis and its complications.[131] The reversal of NASH (with no worsening of fibrosis) or the improvement of fibrosis (without deterioration of NASH) is the endpoint for pre-cirrhotic patients, whereas the main goals in the cirrhotic population are to avoid decompensated cirrhosis, hepatocellular carcinoma, liver transplant, and mortality. Thus, phase IIb and phase III trials require biopsies before and after treatment to establish efficacy, a limitation that could change in the future as newer non-invasive diagnostic methods are validated against biopsy.

No specific agents have so far been approved; nevertheless, pioglitazone and vitamin E are often prescribed off-label, following the results of large randomized studies with histological endpoints. Many more drugs have received or are undergoing evaluation in registered trials.

Pioglitazone

Pioglitazone is an antidiabetic agonist for peroxisome proliferator activated receptor-γ (PPAR-γ), a member of a nuclear receptor family of proteins that modulate several responses, including insulin sensitivity. Its use in NAFLD has been proposed to counteract insulin resistance. Several RCTs and a meta-analysis have consistently shown an improvement in biochemistry and histology after administration of pioglitazone at doses of 30-45 mg/day versus placebo.[132] In the PIVENS trial, which also tested the effects of vitamin E, pioglitazone did not significantly improve NASH (34% *v* 19% for placebo), but aminotransferase concentrations were reduced, as were steatosis and lobular inflammation.[133] In 101 patients with pre-diabetes or type 2 diabetes, pioglitazone (45 mg/day) was particularly effective, achieving the primary outcome (≥2 point improvement in NAS score without worsening of fibrosis) in 58% of cases (versus 17% in controls) and producing resolution of NASH in 51% and change in fibrosis stage (−0.5, 95% confidence interval 0.0 to 0.9, points).[134] A more recent meta-analysis in 197 NASH patients and 195 controls confirmed that pioglitazone was associated with improvement of advanced fibrosis (odds ratio 4.53, 1.52 to 13.52) and NASH resolution (odds ratio 3.51, 1.76 to 7.01).[135] Discontinuation of pioglitazone is accompanied by an abrupt increase in alanine aminotransferase, possibly heralding recurrence of NASH.[136] This makes pioglitazone the long term drug treatment of choice, irrespective of type 2 diabetes. Notably, pioglitazone also has beneficial effects on the cardiovascular system[137] [138]; adverse events include increased body weight and an increased risk of non-osteoporotic fractures.

Vitamin E

Vitamin E has been proposed for the treatment of NAFLD, considering its anti-apoptotic and antioxidant properties, with conflicting results.[132] In the PIVENS trial, at a dose of 800 IU/day, vitamin E significantly improved NASH compared with placebo (49% *v* 19%), as well as reducing steatosis and lobular inflammation, without significant effects on fibrosis (41% *v* 31%; average change in score −0.3 *v* −0.1). Accordingly, US guidelines suggest considering the use of vitamin E in patients with biopsy assessed NASH without diabetes or cirrhosis,[4] a recommendation not shared by the European guidelines.[5]

A very recent trial in biopsy proven NASH with type 2 diabetes compared vitamin E (800 IU/day) with vitamin E and pioglitazone (45 mg/day) or placebo on the primary outcome of reduction in NAS of at least 2 points without worsening of fibrosis. It found that only the combination therapy achieved the target (combination 54%; vitamin E alone 31%; placebo 19%), although both treatments increased the rate of NASH resolution (43%, 33%, and 12%, respectively).[139] Fibrosis did not improve. Regarding safety, the evidence for increased all cause mortality associated with a dose of 800 IU/day, derived from

an old meta-analysis,[140] is no longer supported by data.[141] Vitamin E is the treatment of choice for pediatric NAFLD.[4]

Drugs in phase II and III trials

The evidence on these drugs is summarized below, with details provided in table 4, table 5, and table 6.

Farnesoid X receptor agonists

The farnesoid X receptor belongs to the nuclear receptor superfamily mainly expressed in the liver, intestine, and kidney and, to a lesser extent, in adipose tissues. It regulates a wide variety of target genes critically involved in the control of bile acids, lipids, and glucose (via augmented insulin sensitivity).[154] One of the many consequences of FXR activation is a decreased expression of enzymes involved in de novo lipogenesis; the release of FGF19 from the intestine on bile acid binding to FXR, a major downstream mediator of FXR, potentiates FXR activity and produces additional metabolic effects (PPAR-α activation and suppressed gluconeogenesis), decreased appetite, and increased energy expenditure.[154] Several FXR activating drugs with differing structural characteristics and pharmacodynamic effects are thus under investigation in NAFLD.

Obeticholic acid, a 6α-ethyl derivative of chenodeoxycholic acid, is a first in class selective FXR agonist, originally described for its anticholestatic and potentially broader hepatoprotective properties. The addition of the ethyl group to chenodeoxycholic acid—the natural FXR agonist in humans—multiplies its FXR agonistic activity approximately 100-fold.[154]

A phase IIB clinical trial of obeticholic acid (25 mg/day of oral obeticholic acid versus placebo for 72 weeks) was terminated early after a pre-planned interim analysis at 24 weeks because of overt histological efficacy (≥2 points decrease in NAS, without worsening of fibrosis). Forty six (45%) of 102 patients in the obeticholic acid group had improved liver histology compared with 21/99 in placebo (relative risk 1.9, 1.3 to 2.8).[155]

Obeticholic acid is being evaluated in a phase III trial (REGENERATE, Intercept Pharmaceuticals) at doses of 10 and 25 mg/day versus placebo in NASH patients with fibrosis; liver biopsies were scheduled at screening, at 18 and 48 months, and at the end of study. The results of the interim 18 month analysis in 931 patients with F2-F3 fibrosis have recently been published.[142] Improvement in fibrosis was achieved in 12% of placebo treated patients, 18% in the 10 mg obeticholic acid group, and 23% in the 25 mg obeticholic acid group. The NASH resolution endpoint was not met in the whole intention to treat population (8%, 11%, and 12%, respectively). However, a post hoc analysis showed that approximately twice as many patients treated with 25 mg obeticholic acid achieved NASH resolution compared with placebo, in both intention to treat (23% *v* 12%; relative risk 1.9, 1.4 to 2.8) and per protocol analyses (29% *v* 16%; relative risk 2.2, 1.4 to 3.2).[142] The evaluation is ongoing, to be completed by October 2022. A dossier was submitted to the US Food and Drug Administration (FDA) for regulatory approval on the basis of more than 1700 patients treated with obeticholic acid, but the agency required additional efficacy and safety data to support accelerated approval, while the long term phase continues.[156]

Consistent with other studies of obeticholic acid, dose dependent pruritus, mild to moderate in severity, and increased low density lipoprotein cholesterol, responsive to statin treatment, were the most commonly reported adverse events,[142 154 155]

Table 4 | Therapies for non-alcoholic steatohepatitis (NASH) in phase III development

Drug	Trial code; name (company)	No of patients	Study population	Route of delivery	Time to surrogate endpoint (biopsy)	Primary endpoint	Long term clinical outcome*
Anti-inflammatory, anti-fibrotic							
Obeticholic acid[142] (FXR agonist)	NCT02548351; REGENERATE (Intercept)	2480	NASH with fibrosis F2/F3, NAS ≥4; fibrosis F1 and diabetes, obesity, or inflammation	Oral	72 weeks	≥1 stage improvement of fibrosis without worsening of NASH or NASH resolution without worsening of fibrosis	Time to first event
Cenicriviroc[143] (dual CCR2/CCR5 antagonist)	NCT03028740; AURORA (Allergan)	2000	NASH with fibrosis F2/F3, NAS ≥4	Oral	52 weeks	≥1 stage improvement of fibrosis without worsening of NASH	Time to first event (up to EOS, about 5 years)
Metabolism modulators							
Elafibranor [144] (dual PPAR-α/δ agonist)†	NCT02704403; RESOLVE-IT (Genfit)	2000	NAS ≥4; fibrosis F1/F2/F3 (F1, limited number); BMI ≤45	Oral	72 weeks	NASH resolution (no ballooning, inflammation 0-1, no progression of fibrosis) without worsening of steatohepatitis	Time to first event (up to EOS, about 4 years)
Resmetirom (THRβ agonist)	NCT03900429; MAESTRO-NASH (Madrigal)	2000	NASH with fibrosis F2/F3, high risk F1	Oral	52 weeks	NASH resolution, no worsening of fibrosis. Composite clinical outcome	% patients with >1 event (up to 54 months)
Aramchol (SCD-1 modulator)	NCT04104321; ARMOR (Galmed)	2000	NASH with fibrosis F2/F3, NAS ≥4; overweight/obese; pre-diabetes/T2DM	Oral	52 weeks	NASH resolution, no worsening of fibrosis or ≥1 stage improvement of fibrosis, no worsening of NASH	% patients with >1 event (up to 5 years)

BMI=body mass index; CCR2-CCR5=chemokine receptor 2-5; EOS=end of study; FXR=farnesoid-X receptor; NAS=NAFLD activity score (sum of steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2); PPAR=peroxisome proliferator activated receptor; SDC-1=stearoyl-CoA desaturase modulator; T2DM=type 2 diabetes mellitus; THRβ=thyroid hormone receptor β.
*Long term outcomes include all cause mortality, transplant, and hospital admission due to hepatic decompensation.
†Recent early termination after interim analysis.

Table 5 | Therapies for non-alcoholic steatohepatitis (NASH) in late phase II development

Drug	Trial code; name (company)	No of patients	Study population	Route of delivery	Surrogate endpoint; time to endpoint	Primary endpoint
Metabolism modulators						
Aldafermin[145] (NGM282) (FGF19)	NCT03912532; ALPINE 2/3 (NGM)	152	NASH, fibrosis F2/F3	Subcutaneous	Biopsy; 24 weeks	% patients achieving histological treatment; safety and tolerability
BFKB8488A (bi-specific FGF21/ KLB ab)	NCT04171765; BANFF (Genentech)	260	NASH, fibrosis F2/F3; liver fat ≥8%	Subcutaneous	Biopsy; 52 weeks	NASH resolution without worsening of fibrosis
Icosabutate (structurally enhanced w-3 FA)	NCT04052516; ICONA (NorthSea)	264	NASH, fibrosis F1-F3, NAS ≥4; liver fat ≥10%	Oral	Biopsy; 52 weeks	NASH resolution without worsening of fibrosis
Lanifibranor [146] (pan-PPAR agonist)	NCT03008070; NATIVE (Inventiva)	247	NASH	Oral	Biopsy; 24 weeks	≥2 points reduction of SAF score without fibrosis progression
Licogliflozin (SGLT-1/2)	NCT03205150 (Novartis)	110	NASH, fibrosis F1-F3, elevated ALT or BMI ≥27 (Asian, ≥23); A_{1c} 6.5-10%	Oral	MRI; 12 weeks	Change in ALT
MSDC-0602K[147] (mTOT modulator, Insulin sensitizer)	NCT03970031; MMONARCh (Cirius)	402	NASH, fibrosis+T2D	Oral	Biopsy; 52 weeks	Change in HbA_{1c}; NASH resolution without worsening of fibrosis
Norursodeoxycholic acid [148] (homolog of ursodeoxycholic)	EudraCT2018-003443-31 (Dr Falk)	363	NASH, fibrosis	Oral	Biopsy; 72 weeks	NASH resolution without worsening of fibrosis
Pegbelfermin[149] (PEG-FGF21)	NCT03486899; FALCON 1 (BMS)	160	NASH, fibrosis F3; NAS score ≥1 for each NAS component	Subcutaneous (weekly)	Biopsy; 24 weeks	≥1 stage improvement of fibrosis; no worsening of NASH or NASH resolution; no worsening of liver fibrosis
Efruxifermin[150] (Fc-FGF21 fusion protein)	NCT03976401; BALANCED (Akero Ther.)	80	NASH, fibrosis F1-F3; ≥10% liver fat (MRI-PDFF); NAS score ≥4 (≥1 for each component)	Subcutaneous (weekly)	MRI; 12 weeks. Biopsy; 16 weeks	Change from baseline in hepatic fat fraction assessed by MRI-PDFF
Semaglutide[151] (GLP-1 receptor agonist)	NCT02970942 (Novo Nordisk)	320	NASH, fibrosis F2/F3; NAS ≥4	Subcutaneous	Biopsy; 72 weeks	NASH resolution without worsening of fibrosis
Tirzepatide[152] (dual GLP-1/GIP agonist)	NCT04166773; SYNERGY-NASH (Eli Lilly)	196	NASH, fibrosis F2/F3; BMI ≥27	Subcutaneous	Biopsy; 52 weeks	NASH resolution without worsening of fibrosis
VK2809[153] (THRβ agonist)	NCT04173065; VOYAGE (Viking)	337	NASH, fibrosis F1/F2/F3 NAS ≥4; liver fat ≥8%	Oral	Biopsy; 52 weeks	Change in liver fat
Anti-inflammatory, anti-fibrotic						
CC-90001 (JNK-1 inhibitor)	NCT04048876 (Celgene)	300	NASH, fibrosis <F4; NAS ≥4; BMI 35-45kg/^{m}2	Oral	Biopsy; 52 weeks	≥1 stage improvement of fibrosis
Tropifexor (FXR agonist)	NCT02855164; FLIGHT-FXR(Novartis)	351	NASH, elevated ALT; liver fat ≥10%	Oral	MRI; 12 weeks	Safety and change in ALT and AST

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; FA=fatty acid; Fc=fragment crystallizable region of IgG; FGF=fibroblast growth factor; FXR=farnesoid-X receptor; GIP=gastric inhibitory polypeptide; GLP-1=glucagon-like peptide-1; HbA_{1c}=glycated hemoglobin; JNK=c-Jun N-terminal kinases; KLB=βKlotho; MRI=magnetic resonance imaging; MRI-PDFF=magnetic resonance imaging derived proton density fat fraction; mTOT=mitochondrial target of thiazolidinediones; PEG=pegylated; PPAR=peroxisome proliferator activated receptor; SAF=Steatosis, Activity, Fibrosis; SDC-1=stearoyl-CoA desaturase modulator; SGLT=sodium-glucose cotransporter; T2D=type 2 diabetes; THRβ=thyroid hormone receptor β.

often leading to discontinuation. Combination studies of obeticholic acid with lipid lowering agents are ongoing.

Other FXR ligands are in earlier stages of clinical development. Tropifexor, a non-bile acid derivative FXR agonist with potent activity on fibrosis in experimental NASH models,[157] is being evaluated in a phase II, adaptive design study in NASH (FLIGHT-FXR, Novartis; clinicaltrials.gov NCT02855164). Treatment has been reported to cause a transient increase in serum alanine aminotransferase that declines with time, whereas the expected advantages

Table 6 | Therapies for non-alcoholic steatohepatitis (NASH)-cirrhosis in late stage development

Drug	Trial code; name (company)	No of patients	Study population	Route of delivery	Surrogate endpoint; time to endpoint	Primary outcome
Aldafermin (NGM282) (FGF19)	NCT04210245; ALPINE 4 (NGM)	150	NASH, fibrosis F4 (compensated cirrhosis); liver fat ≥8% (MRI)	Subcutaneous	Biopsy; 48 weeks	≥1 stage improvement in fibrosis, no worsening of NASH; adverse events
Belapectin (galectin-3)	NCT04365868; NASH-CX (Galectin)	162	NASH, fibrosis F4; HVPG ≥6 mm Hg	Intravenous	HVPG; 52 weeks	Change in HVPG
Obeticholic acid (FXR agonist)	NCT03439254; REVERSE (Intercept)	919	NASH, fibrosis F4	Oral	Biopsy; 78 weeks	≥1 stage improvement of fibrosis, no worsening of NASH; or NASH resolution, no worsening of fibrosis
Pegbelfermin (PEG-FGF21)	NCT03486912; FALCON 2 (BMS)	152	NASH, fibrosis F4	Subcutaneous	Biopsy; 48 weeks	≥1 stage improvement of fibrosis, no worsening of NASH
Semaglutide SC (GLP-1 receptor agonist)	NCT03987451 (Novo Nordisk)	69	NASH, fibrosis F4; NAS ≥3; BMI ≥27; stiffness >14 kPa (MRE)	Subcutaneous	Biopsy; 48 weeks	≥1 stage improvement of fibrosis, no worsening of NASH

BMI=body mass index; FGF=fibroblast growth factor; FXR=farnesoid-X receptor; GLP-1=glucagon-like peptide-1; HVPG=hepatic vein pressure gradient; MRE=magnetic resonance elastography; MRI=magnetic resonance imaging; NAS=NAFLD activity score (sum of steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2); PEG=pegylated.

compared with obeticholic acid on pruritus do not seem to be fulfilled.

Another double blind, multicenter, phase IIb RCT is evaluating the safety and efficacy of a combination of tropifexor and cenicriviroc (see below) in patients with biopsy proven NASH and advanced fibrosis (stages F2/F3).[158] Cilofexor, another non-steroidal FXR ligand, is being evaluated alone or in combination with the acetyl-CoA carboxylase inhibitor firsocostat. In a phase II RCT, cilofexor alone was reported to decrease steatosis by more than 30% at magnetic resonance imaging derived proton density fat fraction (MRI-PDFF) in 39% of cases at a daily dose of 100 mg for 24 weeks, in 14% at 30 mg, and in 13% on placebo, without any significant effect on fibrosis, measured by biomarkers and MRS-elastography.[159] When combined with 20 mg firsocostat for 48 weeks in 78 NASH patients with bridging fibrosis or cirrhosis (71 available at follow-up), cilofexor, at a dose of 30 mg, significantly improved necroinflammation (NAS score) and produced a shift toward lower stages of fibrosis, confirmed by reduction in stiffness at transient elastography (≥25% in 45% treated with combination versus 20% in placebo group).[160]

Elafibranor and lanifibranor

Elafibranor is an oral, once daily, first in class drug acting via dual agonism of PPAR-α/δ receptors, with proven efficacy in animal models of NASH and fibrosis. The pivotal phase II study (GOLDEN-505, GENFIT) tested elafibranor (80 and 120 mg versus placebo) over 52 weeks in 276 patients with a diagnosis of NASH and fibrosis (F0-F3); the primary outcome was set as defined by regulatory agencies, with several secondary outcomes.[161] The response rate was higher than that for placebo only in the 120 mg arm (19% *v* 12%; odds ratio 2.31, 1.02 to 5.24) and was more pronounced with increasing baseline severity. In post hoc analysis, exclusion of patients with mild activity led to a significant effect of elafibranor 120 mg versus placebo (odds ratio 3.52, 1.32 to 9.40) in the most severe cases (234 patients with NAS ≥4), doubling the proportion of responders. Both doses improved liver function tests and lipid parameters, as well as fasting serum glucose (−0.98 mmol/L at 120 mg) and glycated hemoglobin (HbA_{1c}; −0.46%) in patients with type 2 diabetes (40% of total). Finally, elafibranor was safe and well tolerated.

Elafibranor was thus moved into a larger, confirmative phase III trial (RESOLVE-IT, GENFIT), to measure four year efficacy. At interim analysis, released on 11 May 2020, the trial did not achieve the expected results. The response rate for the primary endpoint was 19.2% for elafibranor versus 14.7% for placebo, and improvement of at least one fibrosis stage (key secondary endpoint) occurred in 24.5% versus 22.4%, respectively.[144] The trial was terminated early.

Another pan-PPAR agonist (lanifibranor, Inventiva) recently completed a phase IIb, biopsy controlled study in 247 patients with NASH receiving either 800 or 1200 mg/day of active drug versus placebo for six months (NCT03459079). The primary endpoint was a 2 point reduction in the activity part of the SAF score (combining inflammation and ballooning) without worsening of fibrosis; the key secondary endpoints were resolution of NASH without worsening of fibrosis and improvement of fibrosis without worsening of NASH. The results, released on 15 June 2020, show that lanifibranor met both the primary endpoint (41% and 49% for the two doses versus 27% for placebo) and the two secondary endpoints on intention to treat analysis (33% and 45% *v* 19%; 34% and 44% *v* 9%).[146] The drug received FDA designation as breakthrough therapy, intended to expedite the development of drug candidates for serious or life threatening conditions, on 12 October 2020.[162]

Thyroid hormone receptor β agonists

Thyroid hormone receptor β (THR-β) is responsible for regulating specific metabolic pathways in the liver, often impaired in NAFLD, making NAFLD a condition of "hepatic hypothyroidism."[163] Resmetirom (MGL-3196, Madrigal Pharmaceuticals) is a once daily, oral, highly selective agonist of THR-β specifically acting in the liver, without systemic effects (mediated through THR-α in the heart and bone).[163] The mechanism by which resmetirom reduces hepatic fat in NASH is probably dependent on the restoration of normal mitochondrial function and increased β oxidation.

Resmetirom was initially tested in a phase II quadruple blind (participant, care provider, investigator, outcome assessors) RCT in 125 participants with at least 10% liver fat content at MRI-PDFF and biopsy proven NASH (fibrosis F1-F3 and disease activity).[164] The primary outcome was the relative change from baseline in MRI-PDFF. Compared with placebo, resmetirom significantly reduced MRI-PDFF from baseline, both after 12 weeks (least squares mean difference −22.5, 95% confidence interval −32.9 to −12.2) and after 36 weeks (−28.8, −42.0 to −15.7), reduced the markers of liver injury and fibrosis, and reduced disease activity and prompted NASH resolution at liver biopsy in the drug respondent cohort. Resmetirom was generally well tolerated. The most common adverse events were diarrhea and nausea.

Two phase III trials of resmetirom, MAESTRO-NASH and MAESTRO-NAFLD1, are ongoing. MAESTRO-NASH (NCT03900429) is estimated to be completed in 2024. It will include 2000 adults with biopsy proven non-cirrhotic NASH and fibrosis. MAESTRO NAFLD1 (NCT04197479) has recently started and will include 700 adults with MRI-PDFF liver fat fraction 8% or greater and suspected NASH, randomized into four arms: open label, placebo (double blind), resmetirom 80 mg (double blind), and resmetirom 100 mg (double blind). The primary outcome is the incidence of adverse events after 52 weeks of treatment.

A second selective THR-β agonist (VK-2809, Viking Therapeutics) is being tested in a phase IIb trial for 52 weeks in patients with biopsy proven NASH. The results of a daily dose of 5 mg or 10 mg, 10 mg on alternate days, or placebo showed an overall responder rate for more than 30% relative reduction in MRI-PDFF at 12 weeks of 88% versus 17% with placebo.[153] Notably, alternate day administration produced results comparable to the 5 mg/day dose, and lower doses are being tested in phase IIb (1-2.5 mg). The drug was safe and well tolerated, with no serious adverse events reported over the course of the study.

Cenicriviroc

Cenicriviroc is a once daily oral drug that blocks two chemokine receptors, CCR2 and CCR5, involved in inflammatory and fibrogenic pathways. CCRs normally link C-C motif chemokine ligand, overexpressed in liver injury by activated Kuppfer cells or damaged hepatocytes.[165] Cenicriviroc inhibits monocyte recruitment, thereby modulating the hepatic macrophage pool toward less inflammatory and less fibrogenic macrophages.

Cenicriviroc has established anti-inflammatory and antifibrotic activity in animal models of liver disease; in humans, it has been used in HIV infection and, more recently, in NASH. In the phase II CENTAUR study (Tobira Therapeutics),[166] cenicriviroc has been tested in 289 participants with biopsy proven NASH (NAS ≥4), and liver fibrosis (stages F1-F3). The primary endpoint was reached in a similar proportion of patients taking cenicriviroc (n=145, 16%) and placebo (n=144, 19%; odds ratio 0.82, 0.44 to 1.52), and NASH resolution was similarly not different (8% *v* 6%; odds ratio 1.40, 0.54 to 3.63). However, twice as many patients taking cenicriviroc achieved improvement in fibrosis by at least one stage and no worsening of NASH compared with placebo (20% *v* 10%; odds ratio 2.20, 1.11 to 4.35). No differences were seen in body weight and non-invasive biomarkers; safety and tolerability were comparable to placebo.

The two year results have recently been published, with a group of placebo treated patients moved to cenicriviroc: group A (cenicriviroc for two years), group C (placebo for two years), and group B (crossover group). The primary endpoint (≥2 point improvement in NAS with ≥1 point improvement in either lobular inflammation or hepatocellular ballooning, no worsening of fibrosis) was again not met.[143]

A phase III study of cenicriviroc (AURORA; NCT03028740) is ongoing. It will involve up to 2000 adults, aged 18-75 years with NASH and fibrosis F2-F3, who will be followed up for five years. Primary efficacy endpoints will also include time to occurrence of first adjudicated event: death, histopathologic progression to cirrhosis, liver transplant, model of end stage liver disease (MELD) score 15 or higher, ascites, and hospital admission due to liver failure.

The TANDEM trial is a 48 week phase IIb study in 200 adult patients with NASH and biopsy proven fibrosis (F2-F3) that will evaluate the safety and efficacy of a combination of cenicriviroc and tropifexor (LJN452, Novartis) in patients with NASH and fibrosis.[158]

Aramchol

Aramchol is a synthetic lipid molecule obtained by conjugating cholic acid and arachidic acid. Aramchol inhibits the liver enzyme stearoyl coenzyme A desaturase, reducing fatty acid synthesis while increasing fatty acid oxidation, with a lipid lowering effect, mainly via up-regulation of the ABCA1 cholesterol transporter. Aramchol was shown to reduce liver fat in animal models with diet induced fatty liver.[167]

In a phase II randomized, double blind, placebo controlled trial, aramchol (100-300 mg/day) or placebo was administered to 60 patients with biopsy confirmed NAFLD (six with NASH) (NCT01094158). The primary aim was to test whether aramchol would safely and effectively reduce liver fat concentration (MRS assessment). Over three months, liver fat content decreased by 12.6-22.1% in patients given 300 mg/day aramchol, decreased by 2.9-28.2% with 100 mg aramchol, and increased in the placebo group. No serious adverse events were observed.[168]

A second multicenter, randomized, double blind, placebo controlled phase IIb study (ARREST study; NCT02279524) evaluated the efficacy and safety of higher doses of aramchol (400 mg and 600 mg) in NASH patients with overweight or obesity and diabetes or pre-diabetes (247 patients, 52 weeks and 13 week follow-up). The primary outcome was percentage change in intrahepatic triglyceride concentration measured by MRS; histology was a secondary outcome. The study, reported only in abstract form,[169] confirmed that a larger number of patients in the aramchol 600 mg arm achieved resolution of NASH without worsening of fibrosis (16.7% *v* 5% for placebo; odds ratio 4.74, 0.99 to 22.66); biochemistry also improved. A phase III RCT (ARMOR; NCT04104321) is recruiting 2000 patients at high risk of progression. Patients are randomized to receive aramchol 300 mg twice daily or matching placebo. Primary outcomes are the effects on liver histology at 52 weeks and the effects on composite long term outcomes (all cause mortality, transplant, hospital admission due to hepatic decompensation) at five years.

Glucagon-like peptide-1 receptor agonists

GLP-1 is an intestinal hormone released from L cells in the small intestine in response to meals, which has multiple metabolic effects: it stimulates insulin secretion and inhibits glucagon secretion, increases energy disposal, delays gastric emptying, and improves satiety.[170] GLP-1 analogs are commonly used to treat diabetes, and several studies incidentally reported a significant reduction of liver fat in response to treatment.[171]

Liraglutide is a long acting human GLP-1 analog licensed for glycemic control in patients with type 2 diabetes. A meta-analysis based on individual patient data of registration trials with liraglutide (LEAD program, 2241 patients with elevated aminotransferase concentrations) confirmed a significant reduction of liver enzymes in response to treatment, and a trend toward reduced steatosis in the LEAD-2 study. Daily injection of liraglutide for 48 weeks improved NASH histology in a small phase II study (Liraglutide Efficacy and Action in NASH (LEAN) study).[172] Nine (39%) of 23 patients who received liraglutide had resolution of NASH compared with 2/22 (9%) receiving placebo (relative risk 4.3, 1.0 to 17.7). Notably, treatment with liraglutide was associated with significant weight loss (mean difference versus placebo −4.4, 95% confidence interval −7.2 to −1.6, kg). Adverse events included gastrointestinal disorders in 81% of liraglutide treated patients and 65% in the placebo group.

A phase II study of semaglutide, a longer acting, weekly dosing GLP-1 analog, has very recently been published. After 72 weeks of therapy with the highest dosage tested (0.4 mg), 33/56 (59%) patients with fibrosis F2-F3 met the usual primary endpoint of NASH resolution without worsening of fibrosis compared with 10/58 (17%) patients in the control arm. Among patients taking the 0.1 mg and 0.2 mg doses, 40% and 36% achieved the endpoint, respectively. However, the confirmatory secondary endpoint of fibrosis improvement without worsening of NASH was not met. Fibrosis improved by one stage in all arms, with no difference between placebo (33%) and the 0.4 mg semaglutide group (43%).[151] Among patients taking the 0.1 mg and 0.2 mg doses, 40% and 36% achieved the endpoint, respectively. Semaglutide is very effective in term of weight loss; a phase III-IV trial in obesity reported a mean weight loss of 14.9% with semaglutide 2.4 mg/week for 68 weeks versus 2.4% with placebo, and additional weight loss at follow-up (to 17.4%) compared with weight regain in placebo treated patients.[173]

Synergistic effects may be achieved by combining GLP-1 receptor agonists with lifestyle intervention,[174] with gastric inhibitory polypeptide (GIP), or with glucagon receptor agonists. In a phase II, 26 week trial comparing tirzepatide, a once weekly injected GIP/GLP-1 combined agonist, versus placebo and versus dulaglutide, another weekly dosing GLP-1 receptor agonist, tirzepatide showed better effects on several NASH biomarkers.[152] Differences in liver enzymes, keratin-18, procollagen III, and adiponectin were partly explained by the larger weight loss achieved by tirzepatide treatment.

Drugs for selected patients

People with type 2 diabetes constitute a relevant cohort of NASH patients, at higher risk of disease progression and requiring pharmacologic control of their metabolic defects. A few classes of antidiabetic agents have shown significant effects on liver enzymes and surrogate biomarkers of steatosis and fibrosis, potentially reducing the risk of end stage liver disease. Trials with GLP-1 receptor agonists have been discussed above; several cohort studies are also available to support a beneficial effect of long acting GLP-1 receptor agonists,[175] potentially making these drugs the treatment of choice in the presence of NASH and also improving cardiovascular outcomes.

MSDC-0602 (Cirius Therapeutics) is an insulin sensitizer of the thiazolidinedione class, acting through modulation of mitochondrial pyruvate carrier with minimum PPAR-γ activity. It showed no benefit on primary and secondary histological outcomes in the general NASH population but fulfilled some endpoints in the type 2 diabetes subset[147]; accordingly, a specific trial has been planned in patients with NASH, fibrosis, and diabetes (NCT03970031).

Gliflozins, the sodium-glucose cotransporter-2 inhibitors, by blocking glucose resorption from the proximal tubule, promote glycosuria, calorie waste, and weight loss. This possibly translates into reduced lipid burden to the liver. Most approved gliflozins have been tested for their effects on biomarkers of steatosis and fibrosis,[176-178] and other compounds are under scrutiny, but very few histological data are available. A network meta-analysis of 29 RCTs confirmed that gliflozin treatment was significantly associated with weight loss of at least 5% versus placebo (dapagliflozin 10 mg: odds ratio 8.57, 95%credible interval 2.71 to 27.44; empagliflozin 25 mg: 10.20, 4.59 to 28.93).[179] Unfortunately, very few comparative analyses of the effect of different antidiabetic treatments on liver disease progression in NAFLD with diabetes exist.[180]

Other compounds

Several other drugs, not discussed above and acting on different biochemical processes, are under investigation in phase II trials. Among them, nor-ursodeoxycholic acid (1500 mg/day), also being tested in primary biliary cholangitis, showed a reduction of serum alanine aminotransferase versus placebo in a 12 week RCT (mean difference −27.8, 95% confidence interval −34.7 to −14.4) without relevant side effects, but too few data on MRS-PDFF and liver stiffness were available to derive firm conclusions.[148] Much attention has also been paid to an engineered version of FGF19 (aldafermin), to pegylated FGF21 (pegbelfermin), and to long acting efruxifermin, all able to stimulate adiponectin secretion, thus reducing insulin resistance and inflammation, as well as to reduce body weight. Subcutaneous daily aldafermin injection met the primary endpoint of significant reduction of liver fat in a phase II, 24 week study in 78 NASH patients with fibrosis F2-F3 versus placebo. At histology, there was a trend, but no significant differences, toward improvement in fibrosis of more than one stage (38% *v* 18%), as well as NASH resolution with no worsening of fibrosis (24% *v* 9%).[145] On this basis, a study in NASH cirrhosis is ongoing

(ALPINE4; NCT04210245). Pegbelfermin was initially tested in the FALCON1 study, a multidose, 16 week, phase II trial versus placebo. The trial was terminated early, because of overt superiority of the study drug on absolute change in hepatic fat content (MRI-PDFF).[149] On this basis, the drug was moved to phase IIb in NASH cirrhosis (FALCON2 study; NCT03486912). Another engineered, weekly dosing, subcutaneous FGF21 compound (efruxifermin, human immunoglobulin (IgG1) Fc-FGF21 fusion protein) has been investigated in a 16 week, phase II study across the whole spectrum of fibrosis stages (BALANCED study; NCT03976401). The primary endpoint was change in steatosis on MRI-PDFF at 12 weeks. Patients who met the primary endpoint (50/80; only two among controls) were eligible for biopsy at 16 weeks, which showed improvement of fibrosis without NASH worsening in 48% of cases, with 28% achieving improvement by at least two stages.[150] The drug has received priority medicines (PRIME) designation from the European Medicines Agency as a treatment for NASH, and a phase IIb/III, adaptive design RCT in biopsy confirmed NASH patients has been planned, at a weekly dose of 28 mg and 50 mg.

Placebo and risk stratification in clinical trials

Stratification is essential to define the effectiveness of a treatment. Type 2 diabetes has a large effect on the response rate of drugs; as an example, in the CENTAUR study,[166] the primary endpoint was achieved in 20% of patients in the experimental arm versus 10.4% in the placebo arm (odds ratio 2.20); however, the drug was much more effective in patients without diabetes (odds ratio 3.84, 1.26 to 11.7) than in those with diabetes (1.40, 0.59 to 3.35).

Active changes in lifestyle may contribute to the heterogeneous and often high rate of "placebo response," driven by possible modifications in lifestyle during a trial (Hawthorne effect). In a recent systematic review and meta-analysis of placebo groups from 39 histology based RCTs of adults with NASH,[181] activity improved by at least 2 points in 25% (95% confidence interval 20% to 30%) of patients in the placebo groups, and fibrosis, liver fat, and liver enzymes improved in 21%.

A recent article by the Liver Forum highlighted the fact that only 26% of RCTs of drugs in NASH had nutritional counseling and/or exercise recommendations, 22% had undefined recommendations, and 52% did not report such interventions.[182] A similar bias was present in studies involving nutritional counseling and/or physical activity, in which the placebo response was variable.[182] Clinical trials in diabetes and obesity confirm the importance of stable lifestyle before screening, as well as the need for improved delivery and reporting of lifestyle recommendations. The Liver Forum recommends that patients enrolled should be evaluated at screening for current diet and exercise habits, have lifestyle stability before baseline screening, and be individually counseled on improving diet and physical activity and decreasing sedentary behavior; all these practices should be appropriately documented throughout the trial.[182] Changes in body weight and physical activity should be recorded and included in the final analysis to avoid potential biases. Quantification of alcohol intake is also challenging, with consistent variability in drinking patterns within NAFLD thresholds, which is likely to influence the results.[183] Finally, gene polymorphisms associated with NASH (*PNPLA3 I148M* and *TM6SF2 E167K*) are likely to affect trial response.

Follow-up and surveillance

The presence of NASH and significant fibrosis requires systematic follow-up and surveillance, but four intertwined questions are still unanswered, both in community patients and in selected cohorts following a liver biopsy: who should be monitored, who should be responsible for surveillance, by which instruments, and how frequently?

European guidelines suggest that patients at low risk of progression might be reconsidered at two year intervals by surrogate biomarkers and eventually by ultrasonography or transient elastography.[5] This time interval is expanded to three years in the National Institute for Health and Care Excellence guideline.[73] Metabolic improvement is associated with reduced steatosis, measurable by FLI, and largely heralded by weight loss.[184] Imaging modalities for a precise quantification of steatosis (for example, MRI-PDFF) should be limited to research settings.[185]

Surrogate serum markers of hepatic inflammation, including alanine aminotransferase, show an overall correlation with the risk of fibrosis progression in large cohorts but are scarcely predictive of progression/regression on an individual basis. Nevertheless, sustained reduction or normalization of elevated alanine aminotransferase can be considered a clinically meaningful endpoint.[186]

Considering the obvious limitations to an extensive use of liver biopsy, changes in non-invasive biomarkers of fibrosis and transient elastography are currently the best tools to monitor disease progression,[71] although very few data are available on day to day variability and their correspondence with histological changes. A better performance is expected from new biomarkers reflecting fibrogenic activity or by MRE-elastography (15% worsening of liver stiffness on MRE is associated with progression of fibrosis at histology).[187 188]

Monitoring and surveillance of patients with NAFLD need to be tailored to disease severity and resource availability,[5] in a complex network including primary care physicians and other specialists. This will help to detect early hepatic decompensation, prompting treatment and eventually inclusion on the waiting list for transplantation,[189] with limits due to cardiovascular comorbidities.[190-192]

No specific strategies exist for screening for NASH induced hepatocellular carcinoma, excluding

the evidence based procedures for cirrhosis (six monthly ultrasonography),[193] but more than half of hepatocellular carcinomas arise in patients without cirrhosis. Although the incidence is insufficiently high to recommend universal surveillance in patients with non-advanced disease, the lack of systematic surveillance in pre-cirrhotic stages may be the reason for late diagnosis of hepatocellular carcinoma.[60] We need to prospectively acquire information on cohorts of patients with NASH to define patients at high risk who should undergo surveillance at earlier stages.

Guidelines

Table 1 provides a summary of recent clinical practice guidelines, including their differences, strengths, and weaknesses.[4 5 73-75]

Conclusions

Forty years after the original description of NAFLD, much is known about its epidemiology and natural history, its pathogenesis, the underlying genetic background, and the risks associated with disease progression, as well as the costs associated with the disease. The condition substantially affects patients' quality of life, and it is expected to become the principal liver disease in future decades. However, we still lack a satisfactory treatment, and weight loss remains the treatment of choice. A matter of concern is the demonstration that epigenetic drivers and/or obesity in childhood or young adulthood might be linked with the risks of cancer and liver failure in later life,[36 194 195] having accumulation of liver fat as a common mechanism.[63]

The high number of patients cannot be managed by specialists, and only selected cohorts at high risk of progression should be referred to their care. Initial experiences of network healthcare have provided interesting results,[76] and they need to be expanded to larger samples. Meanwhile, accurate profiling of patients with NAFLD will help to dissect different phenotypes to refine drug treatments, as well as to plan sequential treatments based on disease stage.

Preventive healthcare strategies based on food related policies to counteract the epidemic of obesity remain a priority to reduce the burden of NAFLD in the general population. Political commitment and concerted actions of the multiple stakeholders involved in prevention and treatment should be mandatory, but very few European countries have so far defined policies to tackle NAFLD in the community.[196] The proactive involvement of patients' associations is highly recommended to include patient reported outcomes among relevant targets of future large scale randomized and observational studies.[197 198]

GLOSSARY OF ABBREVIATIONS

BMI—body mass index
EASL—European Association for the Study of the Liver
ELF—Enhanced Liver Fibrosis
FDA—Food and Drug Administration
FGF—fibroblast growth factor
FIB-4—Fibrosis-4 Index FLI—
Fatty Liver Index FXR—farnesoid
X receptor
GIP—gastric inhibitory polypeptide
GLP-1—glucagon-like peptide-1
ILI—intensive lifestyle intervention
MRI-PDFF—magnetic resonance imaging derived proton density fat fraction
MRS—magnetic resonance spectroscopy
NAFLD—non-alcoholic fatty liver disease
NASH—non-alcoholic steatohepatitis
NFS—NAFLD Fibrosis Score
PPAR-γ—peroxisome proliferator activated receptor-γ
RCT—randomized clinical trial
SAF—Steatosis, Activity, Fibrosis
THR-β—thyroid hormone receptor β

RESEARCH QUESTIONS

- Which biomarkers or imaging tools are suitable for screening patients at risk and/or track meaningful changes in progression/regression of non-alcoholic fatty liver disease (NAFLD) as part of the natural history of the disease or in response to treatment strategies?
- How can we identify distinct phenotypes on the basis of integrated models of history, histology, and "omics" (genome, metabolome, proteome, and microbiome system medicine), also taking into account collinearity in organ status (liver, heart, and pancreas) and the relation between phenotypes and progression of liver disease?
- Should novel regulatory endpoints be established for drug development and biomarker approval (Food and Drug Administration/European Medicines Agency guidance documents) to overcome the risks connected to liver biopsy and to be replicable in clinical practice?
- How can we build a comprehensive network including primary care physicians and liver, diabetes, and obesity specialists for the long term management of disease, also being sensitive to patient reported outcomes, as well as to increase awareness of NAFLD among healthcare professionals and the community?

HOW PATIENTS WERE INVOLVED IN THE CREATION OF THIS ARTICLE

After email communication, the manuscript was sent for review to the Liver Pool (Federazione Nazionale delle Associazioni di Volontariato per le Malattie Epatiche ed il Trapianto di Fegato) and to FEDER (Federazione Diabete Emilia-Romagna). Their comments concerned the questions of screening criteria for advanced disease and patient reported outcomes. The former is discussed in a specific section, and the latter is dealt with in the conclusion. The same associations will be contacted for the dissemination of the review.

Contributors: MLP and LB searched the literature and drafted parts of manuscript; EB and GM planned the review, drafted parts of manuscript, and critically revised the manuscript; all authors approved the final version. GM is the guarantor.

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