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CLINICAL PRACTICE GUIDELINES

EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease

European Association for the Study of the Liver (EASL) · European Association for the Study of Diabetes (EASD) · European Association for the Study of Obesity (EASO)

Abbreviations

ALT	Alanine transaminase
BMI	Body mass index
CAP	Controlled attenuation parameter
CCR	Chemokine receptor
CK-18	Cytokeratin-18 fragments
CKD	Chronic kidney disease
CT	Computed tomography
CVD	Cardiovascular disease
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
ELF	Enhanced liver fibrosis
F	Fibrosis stage
FIB-4	Fibrosis 4 calculator
FLI	Fatty liver index
HbA_{1c}	Glycosylated haemoglobin A_{1c}
HCC	Hepatocellular carcinoma
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
IFG	Impaired fasting glucose
IR	Insulin resistance
LDL	Low-density lipoprotein
MetS	Metabolic syndrome
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAFL	Non-alcoholic fatty liver
NAFLD	Non-alcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	Non-alcoholic steatohepatitis
NFS	NAFLD fibrosis score
NPV	Negative predictive value
OGTT	Oral glucose tolerance test
PNPLA3	Patatin-like phospholipase domain containing 3
PPAR	Peroxisome proliferator-activated receptor
PPV	Positive predictive value
PUFA	Polyunsaturated fatty acids
RCT	Randomised controlled trial
SAF	Steatosis, activity and fibrosis
T2DM	Type 2 diabetes mellitus
TM6SF2	Transmembrane 6 superfamily 2
UDCA	Ursodeoxycholic acid
US	Ultrasound

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Introduction

The Clinical Practice Guidelines propose recommendations for the diagnosis, treatment and follow-up of non-alcoholic fatty liver disease (NAFLD) patients and are the product of a joint effort by the European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO). They update a position statement based on the 2009 EASL Special Conference [1].

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The data have been retrieved by an extensive PubMed search up to April 2015. The final statements are graded according to the level of evidence and strength of recommendation, which are adjustable to local regulations and/or team capacities (Table 1) [2]. In particular, screening for NAFLD in the population at risk should be in the context of the available resources, considering the burden for the national healthcare systems and the currently limited effective treatments. The document is intended both for practical use and for advancing the research and knowledge of NAFLD in adults, with specific reference to paediatric NAFLD whenever necessary. The final purpose is to improve patient care and awareness of the importance of NAFLD, and to assist stakeholders in the decision-making process by providing evidence-based data, which also takes into consideration the burden of clinical management for the healthcare system.

> **Recommendations**
> - Patients with IR and/or metabolic risk factors (i.e. obesity or metabolic syndrome [MetS]) should undergo diagnostic procedures for the diagnosis of NAFLD, which relies on the demonstration of excessive liver fat (**A1**)
> - Individuals with steatosis should be screened for secondary causes of NAFLD, including a careful assessment of alcohol intake. The interaction between moderate amounts of alcohol and metabolic factors in fatty liver should always be considered (**A1**)
> - Other chronic liver diseases that may coexist with NAFLD should be identified as this might result in more severe liver injury (**B1**)

Definition

NAFLD is characterised by excessive hepatic fat accumulation, associated with insulin resistance (IR), and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a proton density fat fraction (PDFF, providing a rough estimation of the volume fraction of fatty material in the liver) >5.6% assessed by proton magnetic resonance spectroscopy (^{1}H-MRS) or quantitative fat/water selective magnetic resonance imaging (MRI). NAFLD includes two pathologically distinct conditions with different prognoses: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH); the latter covers a wide spectrum of disease severity, including fibrosis, cirrhosis and hepatocellular carcinoma (HCC) (Table 2).

The diagnosis of NAFLD requires the exclusion of both secondary causes and a daily alcohol consumption ≥30 g for men and ≥20 g for women [1]. Alcohol consumption above these limits indicates alcoholic liver disease. The relationship between alcohol and liver injury depends on several cofactors (type of alcoholic beverage, drinking patterns, duration of exposure, individual/genetic susceptibility), rendering simple quantitative thresholds at least partly arbitrary. Specifically, patients consuming moderate amounts of alcohol may be still predisposed to NAFLD if they have metabolic risk factors. Of note, the overall impact of metabolic risk factors on the occurrence of steatosis appears to be higher than that of alcohol in these patients [3]. The definitive diagnosis of NASH requires a liver biopsy.

Prevalence and incidence

NAFLD is the most common liver disorder in Western countries, affecting 17–46% of adults, with differences according to the diagnostic method, age, sex and ethnicity [4]. It parallels the prevalence of MetS and its components, which also increases the risk of more advanced disease, both in adults and in children. NAFLD is also present in 7% of normal-weight (lean) persons [5], more frequently in females, at a younger age and with normal liver enzymes. Their liver disease may nonetheless be progressive [6].

NAFLD incidence has rarely been measured. It was 20–86/1000 person-years based on elevated liver enzymes and/or on ultrasound (US), and 34/1000 per year by ^{1}H-MRS [7].

The need for NAFLD screening in the community has been questioned given the high direct and indirect costs of testing, the low predictive value of non-invasive tests, the risks of liver biopsy and the lack of effective treatments [8]. However, the progressive form of NAFLD (i.e. NASH), particularly when associated with advanced fibrosis, should be identified in patients at risk (age >50 years, type 2 diabetes mellitus [T2DM] or MetS), because of its prognostic implications. Validated cost–utility studies on extensive screening programmes are eagerly awaited. Similarly, although familial clustering occurs, family screening is not generally advisable, with the exception of cases with defined inherited diseases (e.g. lysosomal acid lipase deficiency).

> **Recommendations**
> - All individuals with steatosis should be screened for features of MetS, independent of liver enzymes. All individuals with persistently abnormal liver enzymes should be screened for NAFLD, because NAFLD is the main reason for unexpectedly elevated liver enzymes (**A1**)
> - In subjects with obesity or MetS, screening for NAFLD by liver enzymes and/or ultrasound should be part of routine work-up. In high-risk individuals (age >50 years, T2D, MetS) case finding of advanced disease (i.e. NASH with fibrosis) is advisable (**A2**)

Pathogenesis: lifestyle and genes

A high-calorie diet, excess (saturated) fats, refined carbohydrates, sugar-sweetened beverages, a high fructose intake and a Western diet [9] have all been associated with weight gain and obesity, and more recently with NAFLD. High fructose consumption may increase the risk of NASH and advanced fibrosis, although the association may be confounded by excess calorie intake or by unhealthy lifestyles and sedentary behaviour [10], which are more common in NAFLD [11].

> **Recommendations**
> - Unhealthy lifestyles play a role in the development and progression of NAFLD. The assessment of dietary and physical activity habits is part of comprehensive NAFLD screening (**A1**)

Several genetic modifiers of NAFLD have been identified [12], but a minority have been robustly validated (see electronic supplementary material [ESM] 1 Table 1]). The best-characterised genetic association is with *PNPLA3*, initially identified from genome-wide association studies and confirmed in multiple cohorts and ethnicities as a modifier of NAFLD severity across the entire histological spectrum [13, 14]. Recently, the *TM6SF2* gene has been reported as another disease modifier [15, 16] and may have clinical utility assisting risk stratification for liver-related vs cardiovascular morbidity.

The *PNPLA3* rs738409 variant also confers susceptibility and affects the histological pattern of NAFLD and fibrosis in obese children and adolescents [17]. A NASH risk score based on four polymorphisms has been validated in obese children with increased liver enzymes [18].

> **Recommendations**
> - Carriers of the *PNPLA3* I148M and the *TM6SF2* E167K variants have a higher liver fat content and increased risk of NASH. NAFLD due to these variants is not systematically associated with features of insulin resistance. Genotyping may be considered in selected patients and clinical studies but is not recommended routinely (**B2**)

Liver biopsy

Liver biopsy is essential for the diagnosis of NASH and is the only procedure that reliably differentiates NAFL from NASH, despite limitations due to sampling variability [19].

NAFL encompasses: a) steatosis alone, b) steatosis with lobular or portal inflammation, without ballooning, and c) steatosis with ballooning but without inflammation [20]. The diagnosis of NASH requires the joint presence of steatosis, ballooning and lobular inflammation [20–22]. Other histological features can be seen in NASH, but are not necessary for the diagnosis: portal inflammation, polymorphonuclear infiltrates, Mallory–Denk bodies, apoptotic bodies, clear vacuolated nuclei, microvacuolar steatosis and megamitochondria. Perisinusoidal fibrosis is also frequent, but not part of the diagnostic criteria; the term 'borderline' NASH is confusing, unnecessary and should be abandoned. The prospectively designed FLIP algorithm increases observer agreement and precisely defines the grading of ballooning [22]. 'Burned-out NASH' describes regression of advanced disease (steatosis, inflammation or ballooning) in patients exposed to metabolic risk factors.

The NAFLD Activity Score (NAS) scoring system should not be used for the diagnosis of NASH but rather for the evaluation of disease severity, once the diagnosis has been established by the overall pathological assessment. Although NAS is correlated with aminotransferase and homeostasis model assessment of insulin resistance (HOMA-IR) [23], they have a low prognostic value [24]. The steatosis, activity and fibrosis (SAF) score [22] is an alternative with good reproducibility and provides a more accurate and comprehensive description. Fibrosis staging relies on the Kleiner classification [21] (used in a simplified pattern in SAF) [22].

In children, NASH displays many of the features observed in adults, even though the distribution of lesions may be different. Portal inflammation is a frequent feature, but can also be seen in adults with more severe disease [25]. Hepatocellular ballooning and Mallory–Denk bodies are only sporadically observed in paediatric NASH, and portal-based chronic inflammation is predominant [21]. Based on the distinctive histological pattern, a specific histological score (Paediatric NAFLD Histological Score – PNHS) has been validated for better classification of children with/without NASH [26].

Table 1 Evidence grade used for the EASL–EASD–EASO Clinical Practice Guidelines on NAFLD (adapted from the GRADE system [8])

Grading	Notes	Symbol
Grading of evidence		
High quality	Further research is very unlikely to change our confidence in the estimate effect	A
Moderate quality	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate effect	B
Low or very low quality	Further research is very likely to have an important impact on our confidence in the estimate of effect and may change the estimate effect. Any estimate of effect is uncertain	C
Grading of recommendations		
Strong recommendation warranted	Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost	1
Weaker recommendation	Variability in preferences and values, or more uncertainty: more likely a weak recommendation is warranted	2
	Recommendation is made with less certainty; higher cost or resource consumption	

Non-invasive assessment

Non-invasive markers should aim to: (i) in primary care settings, identify the risk of NAFLD among individuals with increased metabolic risk; (ii) in secondary and tertiary care settings, identify those with worse prognosis, e.g. severe NASH; (iii) monitor disease progression; (iv) predict response to therapeutic interventions. Achieving these objectives could reduce the need for liver biopsy.

Table 2 The spectrum of NAFLD and concurrent diseases

Disease	Subclassification	Most common concurrent diseases
NAFLD[a]	NAFL • Pure steatosis • Steatosis and mild lobular inflammation NASH • Early NASH: no or mild (F0-F1) fibrosis • Fibrotic NASH: significant (≥F2) or advanced (≥F3, bridging) fibrosis • NASH-cirrhosis (F4) Hepatocellular carcinoma[c]	AFLD-Alcoholic fatty liver disease[b] Drug-induced fatty liver disease[b] Hepatitis C virus (HCV)-associated fatty liver (genotype 3)[b] Others[b] • Haemochromatosis • Autoimmune hepatitis • Coeliac disease • Wilson's disease • A/hypo-betalipoproteinaemia, lipoatrophy • Hypopituitarism, hypothyroidism • Starvation, parenteral nutrition • Inborn errors of metabolism (Wolman disease [lysosomal acid lipase deficiency])

[a] Also called Primary NAFLD and associated with metabolic risk factors/components of Metabolic Syndrome:

1. Waist circumference ≥94/≥80 cm for Europid men/women

2. Arterial pressure ≥130/85 mmHg or treated for hypertension

3. Fasting glucose ≥100 mg/dl (5.6 mmol/l) or treated for T2DM

4. Serum triacylglycerol >150 mg/dl (>1.7 mmol/l)

5. HDL-cholesterol <40/50 mg/dl for men/women (<1.0/<1.3 mmol/l)

[b] Also called secondary NAFLD. Note that primary and secondary NAFLD may coexist in individual patients. Also NAFLD and AFLD may coexist in subjects with metabolic risk factors and drinking habits above safe limits.

[c] Can occur in the absence of cirrhosis and histological evidence of NASH, but with metabolic risk factors suggestive of 'burned-out' NASH

Steatosis

Rationale Steatosis should be documented whenever NAFLD is suspected as the primary disease or as a coexisting condition. It also predicts future diabetes mellitus, cardiovascular events and arterial hypertension. In clinical practice, quantification of fat content is not of interest, except as a surrogate of treatment efficacy, and is therefore not generally recommended.

In individual patients, especially in tertiary care centres, steatosis should be identified by imaging, preferably US, because it is more widely available and cheaper than the gold standard, MRI (ESM 1 Table 2). US has limited sensitivity and does not reliably detect steatosis when <20% [27, 28] or in individuals with high body mass index (BMI) (>40 kg/m^2) [29]. Despite observer dependency, US (or computed tomography [CT] or MRI) robustly diagnoses moderate and severe steatosis and provides additional hepatobiliary information, hence it should be performed as a first-line diagnostic test. However, for larger scale screening studies, serum biomarkers are preferred, as availability and cost of imaging substantially impact feasibility (ESM 1 Table 3). The best-validated steatosis scores are the fatty liver index (FLI), the SteatoTest® and the NAFLD liver fat score; they have all been externally validated in the general population or in grade 3 obese persons and variably predict metabolic, hepatic and cardiovascular outcomes/mortality. These scores are associated with IR and reliably predict the presence, not the severity, of steatosis [30]. Another imaging technique, the controlled attenuation parameter (CAP) can diagnose steatosis, but has a limited ability to discriminate histological grades and has never been compared with ^{1}H-MRS-measured steatosis. Also, the data from studies comparing CAP with US are inconclusive. Thus more data are needed to define the role of CAP.

Recommendations
- US is the preferred first-line diagnostic procedure for imaging of NAFLD, as it provides additional diagnostic information (**A1**)
- Whenever imaging tools are not available or feasible (e.g. large epidemiological studies), serum biomarkers and scores are an acceptable alternative for the diagnosis of steatosis (**B2**)
- A quantitative estimation of liver fat can only be obtained by ^{1}H-MRS. This technique is of value in clinical trials and experimental studies, but is expensive and not recommended in the clinical setting (**A1**)

Steatohepatitis, NASH

Rationale The diagnosis of NASH provides important prognostic information and indicates an increased risk of fibrosis progression, cirrhosis and possibly hepatic comorbidities (HCC). It may also prompt a closer follow-up and possibly a greater need for more intensive therapy.

Clinical, biochemical or imaging measures cannot distinguish NASH from steatosis [31, 32]. Cytokeratin-18 fragments (CK-18), which are generated during cell death (M65 fragments) or apoptosis (M30 fragments), have modest accuracy for the diagnosis of NASH (66% sensitivity, 82% specificity) [33, 34]. CK-18 changes parallel histological improvement but do not perform better than alanine transaminase (ALT) in identifying histological responders [35]. To date, non-invasive tests are not validated for the diagnosis of NASH.

Recommendations
- NASH has to be diagnosed by a liver biopsy showing steatosis, hepatocyte ballooning and lobular inflammation (**A1**)

Fibrosis

Rationale Fibrosis is the most important prognostic factor in NAFLD and is correlated with liver-related outcomes and mortality [24]. The presence of advanced fibrosis identifies patients in need of in-depth hepatological investigation, including, on a case-by-case basis, confirmatory biopsy and intensive therapies. Monitoring of fibrosis progression is also necessary at variable time intervals.

Many serum markers have shown acceptable diagnostic accuracy as defined by an area under the receiver operating characteristic curve (AUROC) >0.8 (ESM 1 Table 3) [32]. NAFLD fibrosis score (NFS) and fibrosis 4 calculator (FIB-4) have been externally validated in ethnically different NAFLD populations, with consistent results. NFS, FIB-4, Enhanced Liver Fibrosis (ELF) and FibroTest® predict overall mortality, cardiovascular mortality and liver-related mortality. NFS predicts incident diabetes, and changes in NFS are associated with mortality. The tests perform best at distinguishing advanced (≥F3) vs non-advanced fibrosis but not significant (≥F2) or any (≥F1) fibrosis vs no fibrosis [36]. Importantly, the negative predictive values (NPVs) for excluding advanced fibrosis are higher than the corresponding positive predictive values (PPVs) [36, 37]; therefore, non-invasive tests may be confidently used for first-line risk stratification to

exclude severe disease. However, predictive values depend on prevalence rates and most of these studies have been conducted in tertiary centres where the pre-test probability of advanced fibrosis is higher than in the community.

Among imaging techniques, transient elastography performs better for cirrhosis (F4) than for advanced fibrosis (F3). Elastography has a higher rate of false-positive than false-negative results and higher NPV than PPV [38], hence the ability to diagnose bridging fibrosis or cirrhosis is insufficient for clinical decision-making. The main shortcoming of transient elastography is unreliable results in the presence of high BMI and/or thoracic fold thickness. In a large, unselected European series, up to 20% of examinations had unreliable results [39], mainly in obese NAFLD [38]. The XL probe should be used in these patients to reduce the failure rate, which remains high (35%) [40].

There is no consensus on thresholds or strategies for use in clinical practice when trying to avoid liver biopsy [32]. Some data suggest that the combination of elastography and serum markers performs better than either method alone [41]. Importantly, longitudinal data correlating changes in histological severity and in non-invasive measurements are urgently needed.

> **Recommendations**
> - Biomarkers and scores of fibrosis, as well as transient elastography, are acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis/cirrhosis (**A2**). The combination of biomarkers/scores and transient elastography might confer additional diagnostic accuracy and might save a number of diagnostic liver biopsies (**B2**)
> - Monitoring of fibrosis progression in clinical practice may rely on a combination of biomarkers/scores and transient elastography, although this strategy requires validation (**C2**)
> - The identification of advanced fibrosis or cirrhosis by serum biomarkers/scores and/or elastography is less accurate and needs to be confirmed by liver biopsy, according to the clinical context (**B2**)
> - In selected patients at high risk of liver disease progression, monitoring should include a repeat liver biopsy after at least 5-year follow-up (**C2**)

Non-invasive testing in paediatric NAFLD

The position paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)

Hepatology Committee has recently delineated diagnostic criteria for paediatric NAFLD [42]. In obese children, NAFLD should always be suspected; elevated aminotransferase levels and liver hyperechogenicity deserve further evaluation and the exclusion of other causes of liver disease. Due to the poor sensitivity of these tests in overweight/obese children, non-invasive markers and imaging techniques are the first diagnostic step [43].

> **Recommendations**
> - In children, predictors of fibrosis, including elastometry, acoustic radiation force impulse (ARFI) imaging and serum biomarkers might help reduce the number of biopsies (**B2**)

Common metabolic disorders related to NAFLD

NAFLD is tightly associated with IR not only in the liver, but also in muscle and adipose tissues [44], and also with MetS, defined as the cluster of any three of the following five features associated with IR: impaired fasting glucose (IFG) or T2DM, hypertriglyceridaemia, low high-density lipoprotein (HDL)-cholesterol (gender-adjusted), increased waist circumference (ethnicity adjusted) and high blood pressure [45]. As all components of MetS correlate with liver fat content, independently of BMI, the presence of MetS in any given patient should lead to an evaluation of the risk of NAFLD, and vice versa the presence of NAFLD should lead to an assessment of all components of MetS.

Hepatic triacylglycerol accumulation is accompanied by abnormal hepatic energy metabolism [46] and impaired insulin-mediated suppression of hepatic glucose and very low-density lipoprotein production [47], leading to hyperglycaemia, hypertriglyceridaemia and hyperinsulinaemia. In non-diabetic persons, the product of fasting glucose (in mmol/l) and insulin (in mU/ml), divided by 22.5 (HOMA-IR) can serve as surrogate for IR [48], and is therefore an acceptable alternative to more expensive and time-consuming dynamic testing. Liver disease progression has been associated with persistence or worsening of metabolic abnormalities, including HOMA-IR [49, 50]. However, the validity of HOMA-IR depends on the ability of insulin secretion to adapt to IR, questioning its suitability in overt diabetes. Moreover, the assays for insulin measurements vary widely, and there is no agreement on a threshold defining IR using HOMA-IR.

Recommendations

- HOMA-IR provides a surrogate estimate of IR in persons without diabetes and can therefore be recommended provided proper reference values have been established (**A1**)
- HOMA-IR is of limited use for NAFLD diagnosis in patients with metabolic risk factors. It could confirm altered insulin sensitivity, thereby favouring a diagnosis of IR-associated NAFLD in cases of diagnostic uncertainty (e.g. US-defined steatosis with normal body weight) (**B2**)
- During follow-up, HOMA-IR might help identify patients at risk of NASH or fibrosis progression in selected cases. Improvement of HOMA-IR during weight loss may indicate metabolic improvement that could be beneficial for NAFLD (**C2**)

Obesity

BMI and waist circumference, a measure of visceral adiposity, are positively related to the presence of NAFLD [51] and predict advanced disease, particularly in the elderly [52]. A large proportion of patients with cryptogenic cirrhosis have a high prevalence of metabolic risk factors [53], suggesting that the majority of cases of cryptogenic cirrhosis are 'burned-out' NASH. Common comorbidities of obesity, such as T2DM, and sleep apnoea [54], polycystic ovary syndrome and other endocrine disorders (hypogonadism), further drive NAFLD prevalence and severity.

Importantly, patients with BMI <30 kg/m² (or even <25 kg/m²) but with visceral fat accumulation or dysfunctional adipose tissue can exhibit NAFLD with/without abnormal liver enzymes [44, 55]. The currently used concept of 'metabolically healthy' obese individuals should be considered with caution, given that they may exhibit gene expression similar to those of metabolically altered obese patients, and may have altered liver tests and adverse health outcomes when longitudinally examined [56, 57].

Recommendations

- Follow-up is mandatory in obesity, which is the major phenotype and risk condition for NAFLD, driven by IR, and also increases the risk of advanced disease (**A1**)
- Most lean persons with NAFLD display IR and altered body fat distribution even though they have less severe metabolic disturbance than overweight NAFLD. Follow-up is nonetheless required because of possible disease progression (**B2**)

Diabetes mellitus

T2DM patients are insulin resistant, often obese, dyslipidaemic, display increased liver enzymes [58] and tend to accumulate hepatic fat independently of BMI [59, 60]. The prevalence of NAFLD is also higher in persons at risk of T2DM, defined as a glycosylated haemoglobin A_{1c} (HbA_{1c}) of 5.7–6.4% (38.8–46.4 mmol/mol), IFG (fasting glucose: 100–125 mg/dl [5.55–6.94 mmol/l]) and/or impaired glucose tolerance (IGT; glucose: 140–199 mg/dl [7.77–11.04 mmol/l] at 2 h of the standardised 75 g oral glucose tolerance test [OGTT]). Diabetes risk and T2DM closely associate with the severity of NAFLD, progression to NASH, advanced fibrosis and the development of HCC [4, 61], independently of liver enzymes [6]. Conversely, US-defined NAFLD is associated with a 2–5-fold risk of developing T2DM after adjustment for several lifestyle and metabolic confounders [62]. The standardised 75 g OGTT should therefore be performed in persons with increased diabetes risk [63, 64].

Insulin treatment increases body fat, but it does not appear to promote or worsen NAFLD in diabetes [65, 66]. While acute insulin infusion dose-dependently increases liver fat content in T2DM [67], chronic insulin treatment improves adipose tissue IR and therefore reduces NEFA flux and hepatic fat content.

Recommendations

- In persons with NAFLD, screening for diabetes is mandatory, by fasting or random blood glucose or HbA_{1c} (**A1**) and, if available, by the standardised 75 g OGTT in high-risk groups (**B1**)
- In patients with T2DM, the presence of NAFLD should be looked for irrespective of liver enzyme levels, since T2DM patients are at high risk of disease progression (**A2**)

Diagnostic algorithm and follow-up

The incidental discovery of steatosis should lead to comprehensive evaluation of family and personal history of NAFLD-associated diseases and the exclusion of secondary causes of steatosis. Metabolic work-up has to include a careful assessment of all components of MetS [63]. Similarly, the presence of obesity/T2DM or the incidental finding of raised liver enzymes in patients with metabolic risk factors should prompt non-invasive screening to predict steatosis, NASH and fibrosis (Table 3).

Table 3 Protocol for a comprehensive evaluation of suspected NAFLD patients

Level	Item
Initial	1. Alcohol intake: <20 g/day (women), <30 g/day (men)
	2. Personal and family history of diabetes, hypertension and CVD
	3. BMI, waist circumference, change in body weight
	4. Hepatitis B/Hepatitis C virus infection
	5. History of steatosis-associated drugs
	6. Liver enzymes (aspartate and alanine transaminases [γ-glutamyl-trans-peptidase])
	7. Fasting blood glucose, HbA_{1c}, OGTT, (fasting insulin [HOMA-IR])
	8. Complete blood count
	9. Serum total and HDL-cholesterol, triacylglycerol, uric acid
	10. US (if suspected for raised liver enzymes)
Extended[a]	1. Ferritin and transferrin saturation
	2. Tests for coeliac and thyroid diseases, polycystic ovary syndrome
	3. Tests for rare liver diseases (Wilson, autoimmune disease, α1-antitrypsin deficiency)

[a] According to a priori probability or clinical evaluation

Surrogate markers of fibrosis (NFS, FIB-4, ELF or FibroTest) should be calculated for every NAFLD patient, in order to rule out significant fibrosis (≥F2). If significant fibrosis cannot be ruled out, patients should be referred to a Liver Clinic for transient elastography; if significant fibrosis is confirmed, the final diagnosis should be made by liver biopsy (Fig. 1). All cases with diabetes or diabetes risk should be referred to a Diabetes Clinic for optimal management. Those at increased diabetes risk should be included in a structured lifestyle modification programme. Obesity should prompt the inclusion of the patient in a structured weight loss programme and/or referral to an obesity specialist. Finally, all cases should receive comprehensive cardiovascular disease (CVD) work-up.

The optimal follow-up of patients with NAFLD is as yet undetermined. Risk of progression of both the hepatic disease and the underlying metabolic conditions as well as the cost and workload for healthcare providers need to be considered. Monitoring should include routine biochemistry, assessment of comorbidities and non-invasive monitoring of fibrosis. NAFL patients without worsening of metabolic risk factors, should be monitored at 2–3-year intervals. Patients with NASH and/or fibrosis should be monitored annually, those with NASH cirrhosis at 6-month intervals. If indicated on a case-by-case basis, liver biopsy could be repeated after 5 years.

Natural history and complications

Disease progression

In general, NAFLD is a slowly progressive disease, both in adults and in children, but fibrosis rapidly progresses in 20% of cases [68]. The rate of progression corresponds to 1 fibrosis

stage every 14 years in NAFL and every 7 years in NASH, and is doubled by arterial hypertension [68]. NASH is associated with an increased standardised mortality ratio compared
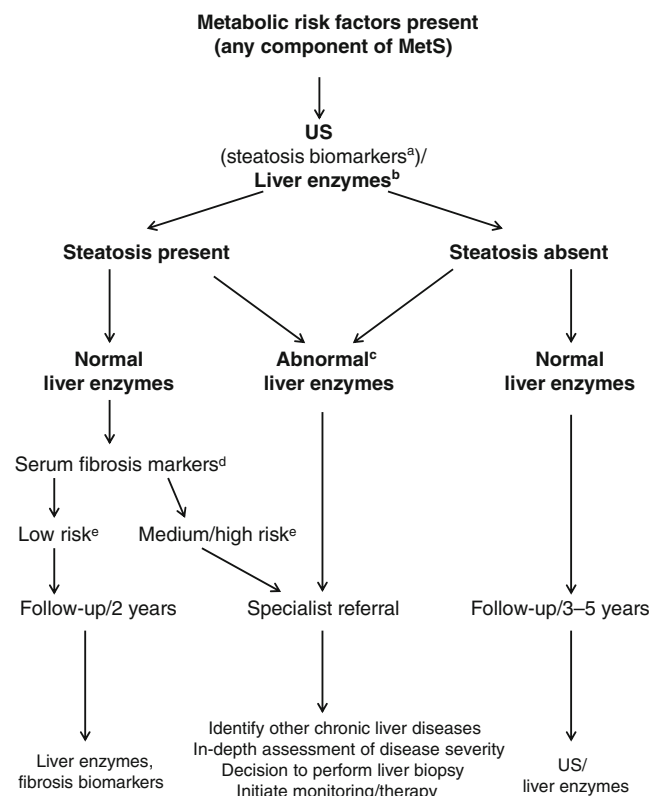
Fig. 1 Diagnostic flow-chart to assess and monitor disease severity in the presence of suspected NAFLD and metabolic risk factors. [a]Steatosis biomarkers: Fatty Liver Index, SteatoTest, NAFLD Fat score (see Tables). [b]Liver tests: ALT AST, γ-glutamyltransferase (GGT). [c]Any increase in ALT, AST or γ-glutamyltransferase (GGT). [d]Serum fibrosis markers: NAFLD Fibrosis Score, FIB-4, Commercial tests (FibroTest, FibroMeter, ELF). [e]Low risk: indicative of no/mild fibrosis; Medium/high risk: indicative of significant fibrosis or cirrhosis (see Tables)

with the general population [69], and liver disease is the third most common cause of death after CVD and cancer. US-diagnosed NAFLD is not associated with increased mortality [70], presumably because progression to NASH and fibrosis is rare for steatosis alone [49, 50].

> **Recommendations**
> • NASH patients with fibrosis associated with hypertension should receive closer monitoring because of a higher risk of disease progression (**B1**)

Paediatric NAFLD is of concern because of the potential for severe liver-related complications later in life [8]. NASH-related cirrhosis has been reported as early as 8 years of age [71].

CVD

The prevalence and incidence of CVD is higher in NAFLD than in matched controls and driven by the association between NAFLD and MetS components [72, 73] (ESM 1 Table 4). CVD is a more common cause of death than liver disease in NAFLD [73]. In most studies, biochemical markers of atherosclerosis (low HDL-cholesterol, high triacylglycerol) or inflammation (high-sensitive C-reactive protein [CRP]), and increased levels of procoagulant/prothrombotic factors are more common in NAFLD than in persons without steatosis [73]. Pre-atherogenic lesions such as increased carotid intima–media thickness; coronary artery, abdominal aortic and aortic valve calcifications; endothelial dysfunction and functional unresponsiveness of the artery wall are more prevalent in NAFLD and are, in some studies, correlated with histological severity. Other defects such as echocardiographic and ECG abnormalities and altered cardiac energy metabolism have also been demonstrated [74]. They are largely independent of traditional risk factors, duration of diabetes, glycaemic control, drug treatment and MetS components. In the general population, US-detected steatosis and its surrogate markers (e.g. FLI) are associated with a higher risk of CVD mortality in the long term [75], and the risk increases further in NASH and in advanced fibrosis [73].

The overall consensus is that CVD should be identified in NAFLD regardless of the presence of traditional risk factors. Conversely, NAFLD screening should be performed in persons at high CVD risk. An association between serum γ-glutamyltransferase (GGT) and CVD incidence has been prospectively established, although it is insufficient for devising follow-up protocols. Notably, CVD and metabolic risk factors

are also reported in adolescents and children with NAFLD [76].

> **Recommendations**
> • Cardiovascular complications frequently dictate the outcome of NAFLD and screening of the cardiovascular system is mandatory in all persons, at least by detailed risk factor assessment (**A1**)

HCC

Large-scale epidemiological studies have repeatedly associated obesity and T2DM with the risk of HCC, and the occurrence of HCC has also been reported in NAFLD/cryptogenic cirrhosis. The cumulative incidence of NAFLD-associated HCC (>10-fold higher in T2DM and obesity) varies according to study population (population-based, natural history vs clinic-based cohorts with/without fibrosis or cirrhosis) from 7.6% at 5 years in persons with advanced fibrosis or cirrhosis to only 0.25% in a larger series followed for 5.6 years [77].

At diagnosis, patients with NAFLD-associated HCC are older than those with non-NAFLD HCC, have more extrahepatic comorbidities, but a lower prevalence of cirrhosis (only two-thirds of cases) (ESM 1 Table 5). NAFLD-related HCC may, however, be diagnosed at more advanced stages, due to less systematic surveillance, and receive less treatment. Conflicting data are reported on survival. At present, NAFLD is the second leading indication for HCC-related transplantation in the USA [78].

The large number of NAFLD cases at risk of HCC makes systematic surveillance largely impracticable. The *PNPLA3* rs738409 C>G gene polymorphism has been associated with an increased HCC risk and might provide patient-risk stratification for tailored HCC surveillance in NAFLD, but it is not yet considered cost-effective (ESM 1 Table 1).

> **Recommendations**
> • Although NAFLD is a risk factor for HCC, which may also develop in the pre-cirrhotic stage, and the risk is further increased by the presence of the *PNPLA3* rs738409 C>G polymorphism, no recommendation can be currently made on the timing of surveillance and its cost-effectiveness (**B1**)

Other extrahepatic disorders

Chronic kidney disease (CKD) can be found in 20–50% of NAFLD patients, particularly in biopsy-proven

Table 4 Randomised controlled trials with histological outcomes in NAFLD

Author, year [ref]	Treatment	Duration	Significant results	Comment
Lindor, 2004 [83]	UDCA 13–15 mg/kg; 70; PL, 74	2 years	Changes in steatosis, inflammation or fibrosis not different between arms	Follow-up biopsies: UDCA, 50; PL, 57. No differences in side-effects between arms
Bugianesi, 2005 [84]	MET 2 g, 55; Vit. E, 28; Diet, 27	12 months	Decreased fat, fibrosis and necroinflammation in MET at follow-up	Vit. E and diet combined as control group. Follow-up biopsies only in metformin non-responders
Belfort, 2006 [85]	PIO 45 mg, 29; counselling, 25	6 months	Improved biochemistry and histology (including fibrosis)	4 cases in PIO and 3 in counselling lost to follow-up
Zelber-Sagi, 2006 [86]	ORL 120 mg ×3, 21; PL, 23 (biopsy, 40)	6 months	Larger weight loss and reversal of steatosis in ORL. No effects on fibrosis	Only 11 cases per arm had biopsy at follow-up
Dufour, 2006 [87]	UDCA 12–15 mg + Vit. E 400 IU, 15; UDCA+PL, 18; PL+PL, 15	2 years	Improved composite histological index with combined treatment. No changes in fibrosis	Only 32 cases with an end-of-treatment biopsy
Ratziu, 2008 [88] FLIRT trial	RSG 8 mg, 32; PL, 31	12 months	Improved steatosis, no differences in fibrosis or necroinflammation	10 cases lost to follow-up (RSG, 7; PL 3); weight gain as side effect of treatment
Aithal, 2008 [89]	PIO 30 mg, 37; PL, 37	12 months	Improved histology (liver injury and fibrosis)	13 patients withdrew; weight gain differences, 3 kg with PIO
Haukeland, 2009 [90]	MET 2.5–3 g, 24; Controls, 24	6 months	No differences in CT-assessed steatosis, biochemistry, histology	Per protocol analysis; 4 dropouts in MET
Harrison, 2009 [91]	Vit. E 800 IU + ORL 120 mg ×3, 25; Vit. E, 25	36 weeks	Similar improvement in steatosis, inflammation and activity scores	Only 41 biopsies at follow-up. Weight loss ≥9% associated with improved histology, independent of treatment
Shields, 2009 [92]	MET 0.5–1 g, 9; Counselling, 10	12 months	No differences in biochemistry or histology	Per protocol analysis, 3 dropouts in counselling
Promrat, 2010 [93]	CBT, 21; Controls, 10	48 weeks	Decreased fat and NAS score	CBT aimed at 7–10 weight loss. Results driven by weight loss
Ratziu, 2010 [94] FLIRT 2 trial	RSG, 53 (RSG-RSG, 25; PL-RSG, 28)	24 months	No further histological improvement beyond one year	Extension of [88]. Only 40 cases available at follow-up (RSG-RSG, 18; PL-RSG, 22)
Sanyal, 2010 [95] PIVENS trial	NASH (no T2DM) PIO 30 mg, 87; Vit. E, 84; PL, 83	96 weeks	Vit. E better than PL but no better than PIO. Both drugs better than PL on steatosis and lobular inflammation, not fibrosis	PIO failed the primary outcome, but Vit. E better than PL on NASH score. Weight gain was a side effect of PIO
Leuschner, 2010 [96]	UDCA 23–28 mg/kg, 95; PL, 91	18 months	UDCA better than PL only in lobular inflammation	NAS score only available in 69 (UDCA) and 68 (PL)
Zein, 2011 [97]	PTX 400 mg ×3, 26; PL, 29	12 months	PTX improved NAS score more than PL. Improved fibrosis (not significant) in PTX	3 dropouts in both groups; no difference in secondary outcomes (liver enzymes, apoptosis, cytokines)
Lavine, 2011 [98] TONIC trial	Paediatric study Vit. E 800 UI, 57; MET 1 g, 57; PL, 58	96 weeks	NAS score improved with PL, no benefit was seen with Vit. E or MET for aminotransferases. Compared with PL, NAS score improved in all groups	Histology was only a secondary outcome
Neuschwander-Tetri, 2015 [99] FLINT trial	OCA 25 mg, 141; PL 142 Trial stopped for superiority: OCA, 102; PL, 98	72 weeks	Early discontinuation for efficacy: improved histology (steatosis, lobular inflammation, ballooning, fibrosis) in 45% OCA vs 21% PL	Increase in LDL-cholesterol and pruritus in 23% of OCA treated cases (sometimes intense, widespread and/or interfering with daily activities)

Table 4 (continued)

Author, year [ref]	Treatment	Duration	Significant results	Comment
Valenti, 2014 [100]	NASH with high ferritin or high iron Phlebotomy + Lifestyle, 21; Lifestyle, 17	2 years	NAFLD activity score (primary outcome) significantly improved. Histology was secondary outcome	Only 19 cases underwent follow-up biopsies
Takeshita, 2014 [101]	EZE 10 mg/day, 17; PL, 15	6 months	Prematurely stopped for EZE adverse events (increased HbA_{1c}). Modest improvement in NAFLD staging and ballooning	Only 16 EZE and 12 PL available for follow-up histology (secondary outcome). Lipid profile and gene expression, suggestive of impaired oxidation of long-chain fatty acids
Sanyal 2014 [102]	EPA-E 1.8 g, 82; EPA-E 2.7 g, 86; PL, 75	12 months	In the 3 arms, 40%, 37%, and 35.9% of cases reached the primary endpoint (NAFLD activity score ≤3, no worsening of fibrosis)	No significant effects on liver enzymes, insulin resistance, adiponectin, keratin 18, high-sensitivity C-reactive protein, or hyaluronic acid
Loomba, 2015 [103] MOZART trial	EZE 10 mg, 25; PL, 25	24 weeks	EZE not better than PL on liver fat (primary outcome, MRI assessment)	No differences in histology or MR-liver stiffness (secondary outcomes)
Argo, 2015 [104]	n-3 PUFA 3 g, 17; PL, 17	1 year	PUFA not better than PL on NAS reduction ≥2 points without fibrosis progression	PUFA led to reduced liver fat by multiple measures, regardless of weight loss
Armstrong, 2015 [105] LEAN programme	LIRA 1.8 mg, 26; PL, 26	48 weeks (extension to 72)	NASH resolution significantly higher with LIRA (39% vs 9% in PL)	NASH resolution, no worsening of fibrosis as primary outcome (follow-up biopsies available in 23 + 22 cases)

C, control arm; CBT, cognitive-behaviour therapy; E, Experimental arm; EPA, Eicosapentaenoic acid; EZE, Ezetimibe; LIRA, liraglutide; MET, metformin; MR, magnetic resonance; PL, placebo; PTX, pentoxifylline; RSG, rosiglitazone

NASH [79]. US-defined NAFLD carries a 1.5- to 2-fold adjusted risk of incident CKD in type 1 diabetes mellitus [80].

NAFLD is also associated with colorectal cancer [81], metabolic bone disease (vitamin D deficiency, osteoporosis) [62, 82] and rare metabolic diseases (lipodystrophies, glycogen storage diseases).

Treatment

Rationale Successful treatment of NASH should improve outcomes, i.e. decrease NASH-related mortality, reduce progression to cirrhosis or HCC. The resolution of the histological lesions defining NASH is now accepted as a surrogate endpoint, particularly in clinical trials. Only a few properly designed randomised controlled trials (RCTs) are available, with improvement/regression of hepatic necroinflammation and/or fibrosis as primary outcomes [83–105] (Table 4).

Diet and lifestyle changes

Rationale Epidemiological evidence suggests a tight relationship between unhealthy lifestyle and NAFLD [106], which makes lifestyle correction mandatory in all patients (Table 5). Of note, daily alcohol consumption up to 30 g (men) or 20 g (women) is insufficient to induce alcoholic steatosis and might even be protective against NAFLD, NASH and fibrosis as compared with total abstinence.

Relatively small amounts of weight loss reduce liver fat and improve hepatic IR [119]. In a pilot RCT of cognitive-behaviour therapy, lifestyle intervention resulted in more weight loss, more frequent resolution of NASH and a borderline higher ($p = 0.05$) reduction in the NAS score [93]. In a post hoc analysis, a weight loss ≥7% was associated with histological improvement. In an uncontrolled 12-month study with 261 paired biopsies, a modest lifestyle-induced weight loss was associated with NASH regression (25% of total cases) without worsening of fibrosis [120].

Pragmatic approaches combining dietary restriction and a progressive increase in aerobic exercise/resistance training [121] are preferable and should be individually tailored. No data are available on their long-term effects on the natural history of NAFLD.

Recommendations

- Structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable in NAFLD (**C2**)
- Patients without NASH or fibrosis should only receive counselling for healthy diet and physical activity and no pharmacotherapy for their liver condition (**B2**)
- In overweight/obese NAFLD, a 7–10% weight loss is the target of most lifestyle interventions, and results in improvement of liver enzymes and histology (**B1**)
- Dietary recommendations should consider energy restriction and exclusion of NAFLD-promoting components (processed foods, and foods and beverages high in added fructose). The macronutrient composition should be adjusted according to the Mediterranean diet (**B1**)
- Both aerobic exercise and resistance training effectively reduce liver fat. The choice of training should be tailored based on patients' preferences to be maintained in the long term (**B2**)

Drug treatment

Rationale Drug therapy should be indicated for progressive NASH (bridging fibrosis and cirrhosis) but also for early-stage NASH with increased risk of fibrosis progression (age >50 years; diabetes, MetS, increased ALT [122]) or active NASH with high necroinflammatory activity [123]. No drug has currently been tested in phase III trials and is approved for NASH by regulatory agencies. Therefore, no specific therapy can be firmly recommended and any drug treatment would be off-label (for reviews see [124–126], Table 4). Safety and tolerability are essential prerequisites for drug treatment, because of NASH-associated comorbidities and polypharmacy, a potential source of drug–drug interactions.

Insulin sensitisers

There is scarce evidence for a histological efficacy of metformin in NASH [84, 90, 92]. The effect of metformin on liver fat is weak, because of its inability to restore serum adiponectin levels in the short-term [127]. Some preclinical data support an anti-tumorigenic activity of metformin on liver cancer [128], while the demonstration of reduced rates of HCC in humans is limited to

Table 5 Elements of a comprehensive lifestyle approach to NAFLD treatment

Area	Suggested intervention	Supportive literature
Energy restriction	• 500–1000 kcal energy defect, to induce a weight loss of 500–1000 g/week	Calorie restriction drives weight loss and the reduction of liver fat, independent of the macronutrient composition of the diet [107]
	• 7–10% total weight loss target	A 12-month intensive lifestyle intervention with an average 8% weight loss leads to significant reduction of hepatic steatosis [108].
	• Long-term maintenance approach, combining physical activity according to the principles of cognitive-behavioural treatment	Hepatic fat increases along with total body fat regain, but most of the beneficial metabolic effects are maintained and progression to T2DM is delayed [109].
Macronutrient composition	• Low-to-moderate fat and moderate-to-high carbohydrate intake	Adherence to the Mediterranean diet has been reported to reduce liver fat on ^{1}H-MRS, when compared with a low fat/high carbohydrate diet in a crossover comparison [110, 111].
	• Low-carbohydrate ketogenic diets or high-protein	
Fructose intake	• Avoid fructose-containing beverages and foods	In the general population, an association has been reported between high fructose intake and NAFLD [9]
Alcohol intake	• Strictly keep alcohol below the risk threshold (30 g, men; 20 g, women)	In epidemiological surveys, moderate alcohol intake (namely, wine) below the risk threshold is associated with lower prevalence of NAFLD, NASH and even lower fibrosis at histology [112–114]. Total abstinence is mandatory in NASH-cirrhosis to reduce the HCC risk [115].
Coffee drinking	• No liver-related limitations	Protective in NAFLD, as in liver disease of other aetiologies, reducing histological severity and liver-related outcomes [116].
Exercise/physical activity	• 150–200 min/week of moderate-intensity aerobic physical activities in 3–5 sessions are generally preferred (brisk walking, stationary cycling)	Physical activity follows a dose–effect relationship and vigorous (running) rather than moderate exercise (brisk walking) carries the full benefit, including for NASH and fibrosis [110, 117, 118]
	• Resistance training is also effective and promotes musculoskeletal fitness, with effects on metabolic risk factors	
	• High rates of inactivity-promoting fatigue and daytime sleepiness reduce compliance with exercise	Any engagement in physical activity or increase over previous levels is however better than continuing inactivity.

retrospective studies [129] and insufficient for evidence-based recommendations.

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR)γ agonists with insulin-sensitising effects. The PIVENS trial compared low dose pioglitazone vs vitamin E vs placebo for 2 years in patients without overt diabetes. Pioglitazone improved all histological features (except for fibrosis) and achieved resolution of NASH more often than placebo [95]. The histological benefit occurred together with ALT improvement and partial correction of IR. Similar results were reported in two smaller and shorter RCTs [85, 89].

Prolonged therapy with rosiglitazone, up to 2 years, did not result in further histological improvement [88, 94], although this was not formally tested with pioglitazone. Side effects of glitazones are of concern: weight gain, bone fractures in women and, rarely, congestive heart failure. Despite the safety and tolerability profile, pioglitazone can be used for selected patients with NASH, particularly in T2DM where the drug has a registered use.

Incretin mimetics, acting on the glucose–insulin interplay have shown favourable results in pre-marketing studies on liver enzymes [130]. A small pilot trial of daily injections of

liraglutide met the histological outcome of NASH remission without worsening of fibrosis [105].

Antioxidants, cytoprotective and lipid-lowering agents

In the PIVENS trial, vitamin E (800 IU/day) improved steatosis, inflammation and ballooning and induced resolution of NASH in 36% of patients (21% in the placebo arm) [95]. Reduced ALT correlated with histological improvement, and histological non-responders did not reduce ALT [131]. In the paediatric TONIC trial [98], vitamin E failed to reduce aminotransferases, steatosis and inflammation but improved ballooning and doubled the rate of NASH clearance vs placebo. These results contrast with previous trials, which were mostly negative in both adults and children. Concerns about long-term safety of vitamin E exist, mainly an increase in overall mortality [132], in haemorrhagic stroke [133] and prostate cancer in males older than 50 [134]. Vitamin E may be used in non-cirrhotic non-diabetic NASH patients but further studies are needed before firm recommendations can be made.

Ursodeoxycholic acid (UDCA) has been investigated in several RCTs, at different doses and for up to 2 years, but only showed some biochemical but no histological improvements [83, 87, 96].

A synthetic farnesoid X receptor agonist, obeticholic acid, improved IR in T2DM [135]. In the phase IIb FLINT trial, a 72-week treatment with obeticholic acid in non-cirrhotic NASH patients, improved all NASH lesions while improving fibrosis [99]. Main issues with safety and tolerability were increased low-density lipoprotein (LDL)-cholesterol and pruritus.

Preliminary data from small or uncontrolled studies suggested that n-3 polyunsaturated fatty acids (PUFA) might reduce liver fat [136], but two trials testing PUFA on histological outcomes were negative [102, 104]. Available data on pentoxifylline and orlistat are limited or inconclusive [86, 91, 97]. Also, data on lipid-lowering drugs are poor; recent trials with ezetimibe were negative [101, 103], whereas statins have not been adequately tested. Their use in NAFLD is safe, with no increased risk of hepatotoxicity, and may even significantly reduce aminotransferases [137].

Promising novel agents with anti-inflammatory, antifibrotic or insulin-sensitising properties (dual PPARα/δ agonists, dual chemokine receptor [CCR]2/CCR5 antagonists and fatty acid/bile acid conjugates) and antifibrotic agents (anti-lysyl oxidase-like [anti-LOXL2] monoclonal antibodies) are also being tested in late-phase RCTs in NASH.

Recommendations

- Pharmacotherapy should be reserved for patients with NASH, particularly for those with significant fibrosis (stage F2 and higher). Patients with less severe disease, but at high risk of disease progression (i.e. with diabetes, MetS, persistently increased ALT, high necroinflammation) could also be candidates to prevent disease progression (**B1**)
- While no firm recommendations can be made, pioglitazone (most efficacy data, but off-label outside T2DM) or vitamin E (better safety and tolerability in the short term) or their combination could be used for NASH (**B2**)
- The optimal duration of therapy is unknown; in patients with increased ALT at baseline, treatment should be stopped if there is no reduction in aminotransferases after 6 months of therapy; in patients with normal ALT at baseline, no recommendations can be made (**C2**)
- Statins may be confidently used to reduce LDL-cholesterol and prevent cardiovascular risk, with no benefits or harm on liver disease. Similarly n-3 polyunsaturated fatty acids reduce both plasma and liver lipids, but there are no data to support their use specifically for NASH (**B1**)

Iron depletion

Hepatic iron accumulation is associated with IR, and iron depletion improves IR [138]. In NAFLD, high ferritin levels are common, in the presence of variable transferrin saturation, independent of gene polymorphisms of familial haemochromatosis. In these patients, a phlebotomy programme to reduce iron stores to near iron deficiency improved the NAS score, without worsening fibrosis [100], but more data are needed.

Paediatric NAFLD

In children, diet and exercise training reduce steatosis, but do not affect ballooning, inflammation and fibrosis [139]. Although several drug-based therapies, such as vitamin E and metformin, and dietary supplementation, including probiotics and docosahexaenoic acid, have shown beneficial effects on ballooning, steatosis and inflammation, fibrotic lesions are refractory to treatment [140] and the long-term outcome of paediatric NASH remains poor [141].

> **Recommendations**
> - Diet and physical activity improve steatosis and hepatic inflammation in paediatric NAFLD, but no beneficial effects on fibrosis have ever been demonstrated. No safe drug treatment has proven effective on fibrosis in paediatric NAFLD (**B1**)

> **Recommendations**
> - Liver transplantation is an accepted procedure in NASH patients with end-stage liver disease, with comparable overall survival to other indications, despite a higher cardiovascular mortality. NASH patients with liver failure and/or HCC are candidates for liver transplantation (**A1**)

Bariatric (metabolic) surgery

In patients unresponsive to lifestyle changes and pharmacotherapy, bariatric surgery is an option for reducing weight and metabolic complications, with stable results in the long term [142]. Surrogate markers indicate that bariatric surgery is effective on NAFLD-associated liver injury, and there is also initial evidence for improved necroinflammation and fibrosis [143]. A recent cohort study with 1-year follow-up confirmed that bariatric surgery-associated weight loss cleared NASH in 85% of patients and improved fibrosis in 34% [144], although the possible benefits should be balanced against peri-/post-operative complications. No solid data on the comparative effects of different bariatric procedures on liver fat are available.

> **Recommendations**
> - By improving obesity and diabetes, bariatric (metabolic) surgery reduces liver fat and is likely to reduce NASH progression; prospective data have shown an improvement in all histological lesions of NASH, including fibrosis (**B1**)

Liver transplantation

NAFLD-associated cirrhosis is among the top three indications for liver transplantation. The 3- and 5-year survival is not different in NAFLD vs no NAFLD; NAFLD carries a higher risk of death from cardiovascular complications and sepsis, whereas the risk of graft failure is lower [145, 146]. The overall mortality is associated with BMI and diabetes, with 50% of cases with BMI >35 kg/m^2 dying within 1 year of transplantation [147]. Transplant failure (10% and 45% at 10 and 20 years, respectively [148]) in obese patients is rarely associated with recurrent NASH cirrhosis (≈2%) [146].

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