Position Paper

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A position statement on NAFLD/NASH based on the EASL 2009 special conference☆

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Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are increasingly relevant public health issues owing to their close association with the worldwide epidemics of diabetes and obesity. NAFLD/NASH is one of the most common chronic liver diseases and increases the 5-year direct and indirect health care costs by an estimated 26% [1]. Although evidence-based clinical practice guidelines for this condition are badly needed, currently not enough evidence is available to formulate guidelines in an unbiased, responsible, and unequivocal way. This position statement summarizes the proceedings of the 2009 EASL Special Conference on NAFLD/NASH and proposes expert opinion for different aspects of the clinical care of these patients.

Definition and classification of NAFLD/NASH

NAFLD designates a condition characterized by excessive fat accumulation (steatosis). NASH defines a subgroup of NAFLD where steatosis coexists with liver-cell injury and inflammation (steatohepatitis).

Primary NAFLD/NASH is associated with insulin resistance (IR) and its phenotypic manifestations. Secondary NAFLD/NASH is rare in adults, is unrelated to insulin resistance or the metabolic syndrome, and is due to a number of medical or surgical conditions or drug intake. Historically, primary NAFLD/NASH required the exclusion of other causes of liver disease (viral, autoimmune, genetic, etc.) and a daily alcohol consumption $\leqslant$20 g in women and 30 g in men, based on epidemiological studies showing that alcohol-induced steatosis can occur above these thresholds [2]. Owing to its increasing prevalence and strong association with the metabolic syndrome [3], it is now recognized that NAFLD/NASH can occur together with other chronic liver diseases and that in some cases (chronic hepatitis C [4], hemochromatosis [5], alcoholic liver disease [6]) this can exacerbate liver damage [7]. This strongly argues for a change in nomenclature (such as metabolic fatty liver disease and metabolic steatohepatitis) which would drop the "negative" definition of "nonalcoholic" and would recognize the likely causal role of IR in NAFLD/NASH.

Epidemiology of NAFLD

The prevalence of NAFLD in the general population assessed by ultrasonography is 20–30% in Europe [8,9] and the Middle East [10], 15% in the Far East [11,12], and 16% in some studies of normal weight subjects without metabolic risk factors [2]. A similar prevalence of 15–25% was documented histologically by older, post-mortem studies [13,14]. A surprisingly high prevalence of histological NAFLD has been described in apparently healthy living liver donors: 12–18% in Europe [15,16] and 27–38% in the US [15,17,18]. With sensitive technique such as MR spectroscopy, 34% of US adults have NAFLD [19]. Interestingly, 39% of newly identified cases of chronic liver disease in a US survey had NAFLD [20] which makes NAFLD/NASH one of the top causes of liver disease in Western countries.

Recent studies in tertiary-care centers, using current histological definitions, have shown a surprisingly high prevalence of NASH among NAFLD cases: 43–55% in patients with increased aminotransferases [21,22], as high as 49% in morbidly obese patients [23,24], and 67% in a subset of patients with incident chronic liver disease [20]. In apparently healthy, living liver donors, the prevalence of NASH ranges from 3% to 16% in Europe [15,16] and from 6% to 15% in the US [15,17,18].

Incidence

The incidence of primary NAFLD in Italy was estimated at 2/100/year [25]; a Japanese study in a selected population reported

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10/100/year [26]. Secondary NASH due to tamoxifen use was estimated at 0.2/100 women/year [27].

Risk factors

The prevalence of NAFLD increases with age, is highest in males between 40 and 65 years [28–31] and is higher in Hispanics and lower in African-Americans [19,20]. Family members of subjects with NAFLD are also at increased risk, independent of age and BMI [32,33]. The commonest cause of NAFLD/NASH is primary NAFLD, associated with IR and its phenotypic manifestations, mainly overweight/obesity, visceral adiposity, type 2 diabetes, hypertriglyceridemia and arterial hypertension [3,34,35]. A causal association has been suggested by longitudinal studies showing a chronological association between the progression of the metabolic syndrome and the occurrence of NAFLD [36,37].

Hepatic complications of NAFLD/NASH

Fibrosis and fibrosis progression

In retrospective series from tertiary referral centers, bridging fibrosis is seen in 25–33% of NASH patients at diagnosis, including cirrhosis in 10–15% [38]. NASH is by far the commonest cause of fibrosis and cirrhosis in patients with unexplained increased ALT [21]. A micromorphometry study has suggested that NASH has a fibrotic potential similar to that of chronic hepatitis C after adjustment for fibrotic confounders [39]. Independent predictors of fibrosis are mainly age >45–50 and diabetes but also BMI >28–30 kg/m^2, hypertension, and the degree of IR [40–42]. Advanced fibrosis can coexist with normal aminotransferases [43,44].

Progression of fibrosis has been demonstrated in retrospective series, raising significant methodological issues [45], in particular whether the observed changes are within the range of what can be expected from mere sampling variability [46]. Pooled data [47–51] have shown that improvement occurs in only 21% of patients with progression in 38% (with some progressing by two stages or more or towards cirrhosis). The strongest predictor of fibrosis progression is necroinflammation on the initial biopsy [52]. Rarely, progression of fibrosis may occur in steatosis only [51,53], presumably due either to concurrent non-specific inflammation (insufficient for a steatohepatitis diagnosis) [38] or to missed lesions of steatohepatitis due to sampling variability.

End-stage NASH is an underecognized cause of cryptogenic cirrhosis [54] mainly because steatosis and liver-cell injury can disappear at this stage [39,55]. Past exposure to metabolic risk factors (Table 1) is the key to diagnosis: at least one major risk factor, being overweight or having diabetes, should be present together with hypertension, dyslipidemia, or atheromatosis. Using these criteria 30–75% of cryptogenic cirrhosis can be attributed to burned-out NASH [54,56–59].

Table 1. Metabolic risk factors.

• **Body mass index** >25 kg/m^2 and/or
• **Waist circumference** >94 cm in men, 80 cm in women (Caucasians)
• **Arterial hypertension** >135/85 mmHg
• **Fasting serum glucose** >6.1 mmol/L
• **Serum triglycerides** >1.7 mmol/L
• **HDL-cholesterol** <1 mmol/L (men); <1.3 mmol/L (women)
• **Serum ferritin** >350 μg/L
• **First degree relatives of individuals with obesity and/or diabetes**

Clinical outcomes

Cirrhosis complications. Liver failure is often (30–51%) the first presentation of patients with cirrhotic NASH [60,61] and occurs after 7–10 years in 38–45% of cirrhotic cases [61,62] although available data, all retrospective, are subject to lead-time bias. Causes of death are liver failure, sepsis and variceal hemorrhage, or hepatocellular carcinoma (HCC) [60,61]. The latter is often diagnosed at a late stage [51,59,61,63], and may occasionally occur in non-cirrhotic NASH [64]. Obese or diabetic patients have an increased risk of HCC [65,66] even in association with other chronic liver diseases [67,68].

Survival. Isolated steatosis does not increase overall or liver-related mortality [51,69]. Long-term follow-up studies have shown that NASH increases overall mortality by 35–85% compared to the age and sex-matched general population [51,70,71]. Liver-related mortality is increased 9–10-fold [51,71] with cirrhosis an independent cause of death ranking 3rd vs. 13th in the general population [22,70,71]. This has been confirmed in pediatric series. Cardiovascular mortality is increased two fold in NASH patients [51].

Liver transplantation. The proportion of patients with NASH among those undergoing liver transplantation has steadily increased over the recent past: from 0.1% between 1995 and 2000 to 3.5% in 2005 according to the UNOS database. Some of the patients with cryptogenic cirrhosis should be added to these: in one series one-third of these patients had histological signs of NASH on a detailed histological review of the graft [56]. If half of the patients with cryptogenic cirrhosis have burned-out NASH (based on histology and exposure to metabolic risk factors), then around 7% of liver transplants in the US are performed for NASH [72]. Importantly, this is an underestimate of the proportion of NASH progressing towards end-stage liver disease, as many patients are no longer listed for liver transplantation because of older age and associated comorbidities (mainly obesity, complications of diabetes, or malignancies) (see Tables 2–5).

Extra-hepatic complications of NAFLD/NASH

Beyond damage to the liver, steatosis can also worsen and/or induce IR, worsen glycemic control in patients with type 2 diabetes, and predict subsequent development of the metabolic syndrome; it is also associated with increased cardiovascular risk and events and with essential arterial hypertension. In other endocrine disorders (polycystic ovary syndrome, hypothyroidism, and panhypopituitarism) liver fat is merely related to underlying IR [72–76] without worsening it.

Liver fat and IR

There is a strong relationship between the amount of hepatic fat and impaired insulin action, independent of global or regional adiposity [77–79]. Excessive liver fat is associated with hepatic but also muscle and adipose tissue IR [77,78] and it correlates with all components of the metabolic syndrome [80,81]. Even in healthy, normal weight individuals, liver fat is associated with several features of IR, independent of BMI and intra-abdominal obesity [80]. Therefore the hepatic fat content could identify IR patients who might not be detected by a standard clinical evaluation [77].

Table 2. Epidemiological burden and risk factors.

• NAFLD/NASH is strongly associated with hepatic and systemic insulin resistance and is one of the hepatic complications of the insulin resistance syndrome.
• In the general population of industrialized countries, NAFLD/NASH is present in at least 20% of individuals. The prevalence is much higher in patients with metabolic risk factors. NAFLD/NASH is currently the most frequent cause of incident chronic liver disease. It can coexist with other chronic liver diseases, with some data showing that the association increases fibrotic severity.
• The prevalence of NASH needs to be further defined. In selected groups of healthy individuals, such as living related donors, the prevalence of NASH is as high as 3–16%. In tertiary-care centers the proportion of histologically defined NASH among NAFLD cases ranges from 40% to 55%.

NAFLD as a precursor of the metabolic syndrome (MS)

Although steatosis could be a mere consequence of IR, a causal role in the genesis or worsening of IR has been suggested. Liver fat reduces insulin clearance resulting in hyperinsulinemia, a feature of pre-diabetes [82]. Ultrasonography-diagnosed NAFLD independently increases the risk of incident diabetes 2.5-fold. Follow-up of NAFLD patients showed that steatosis precedes full-blown complications of IR such as diabetes, arterial hypertension or dyslipidemia [70], although this might not be independent of the level of IR or of visceral adiposity [83]. In the general population, high aminotransferases (a surrogate marker for NAFLD) increased the long-term risk of incident diabetes, MS and cardiovascular events [84]. Finally, on a fat-enriched diet, steatosis and hepatic IR occurred earlier than peripheral IR suggesting that IR in the liver is the primary defect in the development of IR associated with obesity [85,86]. Therefore, monitoring of NAFLD patients for metabolic complications and therapeutic interventions aimed at reducing liver fat should be evaluated in future studies.

Liver fat and diabetes control

Even at the stage of full-blown diabetes, the amount of liver fat influences the severity of IR. Patients with type 2 diabetes and steatosis have substantially more IR than those without steatosis [87]; they also have more dyslipidemia and circulating inflammatory markers [87]. Steatosis predicts, to a large extent, the amount of insulin needed for glycemic control [88]. Future studies need to specifically address whether reducing liver fat improves glycemic control in patients with type 2 diabetes.

NAFLD and cardiovascular disease (CVD)

Steatosis is associated with an increased prevalence [89,90] and incidence of cardiovascular disease (non-fatal CVD events [91] and CVD mortality [92]). The association is usually independent of classic cardiovascular risk factors and, in a few cases, of the metabolic syndrome [93,94]. In biopsy-proven NAFLD, steatosis was associated with increased carotid artery intima-media thickness and with carotid plaques [93]; moreover, significant carotid atherosclerosis occurred 5–10 years earlier in subjects with NAFLD [95] than in those without. Biochemical surrogates of NAFLD (GGT and ALT) predicted incident coronary artery disease, stroke, and cardiovascular disease [94,96]. In diabetics NAFLD further increases the risk of incident CVD and of diabetes complications [97,98]. Interestingly, endothelial dysfunction, an early proatherogenic lesion is also associated with NAFLD, and is more advanced in NASH than in bland steatosis [99].

Diagnostic procedures and strategies

Non-invasive diagnosis of liver injury

Non-invasive markers for liver injury in NAFLD should be developed to facilitate screening for at-risk patients and for follow-up of treated or untreated patients. Future trials should optimize non-invasive diagnostic strategies so that the selection of patients for drug therapy becomes possible even when liver biopsy is not routinely available.

Serum markers for liver injury

Fibrosis. Proprietary tests validated on larger cohorts (FibroTest [100], ELF panel [101], and FibroMeter fatty liver [102]) as well as more simple clinical scores are available [40,103,104]. Most can be used to distinguish between advanced and minimal/no fibrosis and a few provide a fibrosis stage equivalent [100,102]. The diagnostic performance appears to be similar to that in hepatitis C. Additional full reports of independent validation are needed.

Steatohepatitis. Diagnosing steatohepatitis is useful for prognosis and management: it identifies patients at risk for fibrosis progression and therefore justifies more intensive counseling on diet and lifestyle and allows the selection of patients for pharmacological therapy [105]. Two serum markers, NASHTest [106] and CK-18 [107] are validated on larger or on multicentric studies, and one in a smaller series, NASH Diagnostics [108]. CK-18 is a promising marker but its diagnostic performance alone could

Table 3. Hepatic and extra-hepatic complications.

• Fibrosis progression is absent or minimal in isolated steatosis but has been documented in retrospective series in more than one-third of NASH patients.
• End-stage NASH is a frequent cause of cryptogenic cirrhosis and should be diagnosed on past or present exposure to metabolic risk factors when histological signs are lacking.
• NASH significantly increases overall and liver-related mortality; cirrhosis, neoplasia, and cardiovascular disease are the main causes of death in NASH patients.
• Hepatocellular carcinoma can occur in cirrhotic NASH, thereby justifying monitoring strategies. Observations of HCC before the cirrhotic stage need to be further documented as this could impact on patient monitoring.
• Steatosis could be a contributing factor to insulin resistance. Steatosis predicts the development of individual features of the metabolic syndrome and future cardiovascular disease. All patients with NAFLD should be screened for cardiovascular risk, and assessment should be periodically repeated (1–2 years) depending on the clustering of risk factors.

Table 4. Diagnostic strategies and screening.

• Screening for NAFLD/NASH is not recommended in the general population; it is recommended in patients with metabolic risk factors and/or well characterized insulin resistance.
• NASH is consensually defined histologically by the association of steatosis, hepatocellular injury, and inflammation. Grading systems for longitudinal changes have been proposed for follow-up and need to be validated in untreated and treated populations.
• There is a significant need for the non-invasive quantification of fibrosis in order to facilitate screening of the large number of patients at risk. The association of serum markers with an imaging method (elastometry) is recommended in order to restrict biopsy to indeterminate or discordant results or those predicted to have advanced fibrosis.
• A non-invasive diagnosis of steatohepatitis is warranted for the identification of patients at risk of progression. The relevance of the non-invasive quantification of steatosis to patient management should be further studied.

be suboptimal [108] and it is confounded by the amount of fibrosis [107].

Steatosis. Tests that *predict* steatosis would be useful if they had a higher sensitivity than conventional imaging. Tests that *quantify* steatosis might be clinically useful for monitoring early changes induced by therapy and, although not proven, for predicting incident metabolic complications of steatosis or diabetes control. Available tests cannot be compared for their diagnostic performance as they have been validated against different standards: ultrasonography [109], liver biopsy [110], or MRI [82]. The FLI test predicts steatosis and could be useful for large-scale screening instead of ultrasonography. SteatoTest [110] and the NAFLD score [82] have a higher sensitivity than ultrasonography and can also quantify steatosis. Only SteatoTest and the Fatty Liver Index have been independently validated [109–112].

Imaging

Ultrasonography, computed tomography (CT), and MRI can identify histological steatosis higher than 20–30% [113] but not steatohepatitis or the degree of fibrosis [114]. Despite lower sensitivity and specificity than CT, ultrasonography is an acceptable first-line screening procedure for NAFLD in clinical practice. Quantification of steatosis by ultrasonography [115] is not reliable since it is operator-dependent and has a low reproducibility. MRI and MR spectroscopy reliably quantify steatosis and measure regional fat depots. However, they are sequence dependent hence standardization for sequence characteristics between centers is necessary, and cost and availability are limiting [116,117]. Other techniques, especially for quantifying fibrosis, such as diffusion-weighted imaging or MR elastography are promising but still experimental.

In selected patients with NAFLD, measurement of liver stiffness by transient elastography has a diagnostic performance for fibrosis close to that in hepatitis C [118,119] although the cut-offs for fibrosis stages are insufficiently defined. Steatosis marginally increases stiffness (by 1 kPa) [120] as does inflammatory activity [121]. Although attractive because of its simplicity, immediate results and reduced sampling error, there are significant limitations. BMI is a major predictor of failure rate (25% above 30 kg/m^2, 41% above 35 kg/m^2) [122], usual values in non-obese healthy individuals without the metabolic syndrome can be as high as 8 kPa [122], and increased liver stiffness can be seen without fibrosis in different conditions [123–125]. New probes designed for obese individuals are currently being tested.

Measurement of IR

The gold standard for the quantitative measurement of insulin sensitivity, the "euglycemic glucose clamp technique" [126], is expensive and time-consuming, therefore surrogate markers are useful. Waist circumference is well correlated with IR [127]. The product of glucose and insulin, calculated through the Homeostasis Model Assessment (HOMA) [128] or the Quantitative Insulin Sensitivity Check Index (QUICKI) [129] is a simple method, although assay variability for insulin requires "normal" values to be defined for each laboratory. Other methods are based on fasting values, mainly indicative of hepatic sensitivity, or on the dynamics of glucose and insulin in response to an oral glucose tolerance test [130,131], indicative of hepatic and peripheral insulin sensitivity. Finally, insulin sensitivity on lipid metabolism may be assessed in the fasting state as the ratio of triglyceride to HDL-cholesterol levels [132].

Liver biopsy

The histological definition of adult NASH is based on a combination of three lesions (steatosis, hepatocellular injury, and inflammation) within a characteristic topographical distribution (mainly centrilobular, zone 3 of the acini). Steatosis is a prerequisite for the diagnosis of NAFLD, with the exception of cirrhotic disease where it can be absent. The minimal threshold is 5% of hepatocytes containing fat droplets. Signs of hepatocellular injury are cytoplasmic clarification and ballooning of liver cells (a cardinal, required feature) with or without acidophil bodies or spotty necrosis [133]. Inflammation, either lobular or portal is composed of mixed inflammatory cells and is of mild intensity. Both are part of the NASH spectrum with portal inflammation present in severe/advanced cases of NASH and lobular inflammation nearly universal [134]. Polymorphonuclear infiltrates and Mallory–Denk bodies, which can be seen in NASH, are not required for the diagnosis. Isolated steatosis or steatosis with lobular inflammation

Table 5. Management of NAFLD/NASH.

• Weight-loss diet and exercise are first-line therapeutic measures in all overweight patients with NAFLD and insulin resistance. Statins and other drugs for insulin resistance-related comorbidities are not contraindicated in patients with NAFLD/NASH, as the risk for hepatotoxicity does not seem to be increased in patients with steatosis.
• In addition to diet and lifestyle measures and depending of the fibrosis stage, patients with NASH might benefit from pharmacologic therapy in order to reverse or slow progression of liver disease. To date there is some evidence for a beneficial effect of glitazones and vitamin E on biochemical and histological parameters, although the benefit on fibrosis is unproven.
• Treatment of NASH is an unmet medical need; placebo-controlled trials with histological end-points are recommended for registration purposes. Post-approval, long-term safety assessment is essential due to associated comorbidities in many NASH patients.

without signs of hepatocellular injury are both part of the wider spectrum of NAFLD but do not qualify as NASH, mainly because of their different outcome [53].

As in all chronic liver diseases, fibrosis may or may not yet be present and therefore it is not part of the definition of NASH. Perisinusoidal fibrosis is a characteristic feature of NASH and current staging systems incorporate both perisinusoidal and portal fibrosis [135]. There is no widely accepted grading classification for NASH. The NAS score is the unweighted sum of steatosis, ballooning and lobular inflammation [135] and was designed primarily to capture treatment-induced histological changes. It can be used for grading purposes, but it should not be used for the diagnosis of NASH [135]. All these grading and staging systems have not yet been sufficiently validated for use by general pathologists.

A distinct histological pattern has been described in two specific patient populations. In children, NASH is characterized by portal inflammation and fibrosis, azonal steatosis and infrequent ballooning or perisinusoidal fibrosis [136]. In bariatric surgery patients NASH is characterized by isolated portal fibrosis and azonal steatosis [42,133,137].

As in other chronic liver diseases sampling variability is a limitation of liver biopsy in NAFLD [138]. Inflammatory lesions and ballooning are highly prone to sampling error as are fibrosis and steatosis which can result in misdiagnosis or understaging [138,139]. There is no safe threshold for eliminating sampling variability [140]; by analogy with other chronic liver diseases, a core fragment of a minimum of 15 and preferably 25 mm is desirable [139].

Diagnostic strategies for NASH

Many individuals at risk for NAFLD/NASH seek medical attention outside the Hepatology clinics and therefore it is important to establish whether and in what settings screening or case finding [141] for NASH is deemed necessary (see "Case finding"). Conversely, when patients with suspected NAFLD/NASH are addressed for hepatological investigations, the procedures to be performed need to be need to be defined on an individualized basis (see "Individual diagnostic strategies in clinical practice"), in particular the indications for liver biopsy.

Case finding

Screening or case finding of NASH [141] aims at diagnosing advanced liver disease, defined as NASH with bridging fibrosis or cirrhosis. Beyond the prognostic information it provides this may also change patient management including specific monitoring strategies, a stricter enforcement of diet and lifestyle measures, or the use of liver-targeted pharmacologic therapy.

Premises:

(1) In the general population, there are currently insufficient data on the prevalence of NASH, NASH-related mortality and on whether diagnosing NASH may change outcomes and be cost-effective.
(2) NASH is strongly linked to IR and should be considered part of the phenotypic complications of IR and the metabolic syndrome. This implies that patients who come to medical attention for IR (i.e. obesity, type 2 diabetes, dyslipidemia polycystic ovarian syndrome, lipodystrophy, or acanthosis nigricans) are at risk of NASH.
(3) Transaminases are not a sensitive test for NAFLD. However, in patients with IR and increased ALT [142], the prevalence of NASH and advanced fibrosis [21] may be higher than in the overall obese or diabetic population.
(4) Due to its high prevalence, NAFLD can occur in patients with other chronic liver diseases. In chronic hepatitis C, and possibly alcoholic liver disease and hemochromatosis, NAFLD (or at least its risk factors) can worsen fibrosis.

Based on the current literature and on what has been discussed during this conference, we suggest the following:

1. General population. Screening for advanced liver disease is not recommended as per premise #1. Additional studies are needed to delineate NAFLD risk factors, its potential as an independent predictor of cardiovascular mortality and NASH-related mortality.
2. In patients who come to medical attention because of IR (as defined in premises #2–3) case finding of advanced liver disease may be performed based on the high prevalence of NASH, the adverse clinical outcomes and the potential changes in patient management. Liver function tests and liver ultrasound may be performed. In patients with increased ALT or with steatosis at ultrasound we suggest that non-invasive methods to evaluate fibrosis be the first-line procedure. At best, a serum and an imaging procedure may be used. Liver biopsy may be restricted to cases where both non-invasive methods suggest advanced fibrosis and to cases with indeterminate or discordant results, thus deemed insufficient to exclude advanced fibrosis. In patients with both increased ALT and steatosis at ultrasound (at higher risk for advanced liver disease, premise #3), liver biopsy could be the first-line procedure until extensive independent validation of non-invasive methods becomes available.
3. Patients with chronic liver diseases other than NAFLD may be screened for metabolic risk factors, IR, and steatosis at ultrasound. If all these are present, we suggest that liver biopsy be performed to assess concurrent NAFLD, as data on non-invasive methods in patients with concurrent liver diseases are lacking.
4. During elective surgical procedures, such as anti-obesity surgery (high risk of NASH and of unsuspected cirrhosis) and cholecystectomy (shared risk factors between NAFLD and cholelithiasis), we suggest that a liver biopsy be performed.

Individual diagnostic strategies in clinical practice

In patients referred for probable NAFLD to the hepatologist, liver biopsy should be performed based on an individualized decision rather than rigid guidelines. Liver biopsy provides both diagnostic and prognostic information on fibrosis and potential for progression. Liver biopsy should not be performed in patients with recent weight change. In patients with stable weight and lifestyle, a watchful period (4–6 months) is useful, aimed at enforcing diet and lifestyle measures, unless previous structured attempts were unsuccessful. If these measures result in weight loss with ALT normalization and a reduction in IR, liver biopsy can be postponed while ALT and non-invasive markers of fibrosis are monitored. If not, liver biopsy should be considered after balancing the risk for advanced fibrosis (age, diabetes, degree of IR), patient

motivation, and competing comorbidities. Non-invasive fibrosis tests, ideally combining serum and imaging methods, may be used to avoid the biopsy, when both suggest the absence of significant fibrosis. New markers should be developed for early identification of patients at risk of progression.

Pathogenesis

Is steatosis still the first hit?

The 'two hit' model of NASH pathogenesis, suggested that the first "hit" is the development of steatosis sensitizing the liver to the second "hit" – oxidative stress and cytokines – leading to the development of necroinflammation and ultimately fibrosis and cirrhosis [143]. This hypothesis has been challenged by recent data suggesting that mechanisms that can drive disease progression can also induce steatosis. Oxidative stress [144] and gut flora/cytokines [145] can induce steatosis as well as necroinflammation and fibrosis. Free Fatty Acids (FFA) can initiate hepatocyte apoptosis [146] in addition to being esterified to triacylglycerols. Endoplasmic stress can also lead to steatosis, oxidative stress and apoptosis [147]. Since all these mechanisms are important in obesity and IR, it would seem likely that they are the true "first hits" leading to increased hepatic FFA flux and oxidative-, ER-, and cytokine-mediated stress that result in both steatosis and progressive liver damage in susceptible individuals. Steatosis should therefore be considered part of the liver's early "adaptive" response to stress, rather than a first hit in disease progression. Accordingly, while in some situations its severity may act as a biomarker of ongoing injurious and fibrotic mechanisms resulting in disease progression, it should not be considered a therapeutic target. Instead attention should be focused on the mechanisms of cellular injury and fibrosis – the "second hits".

Mechanisms of fibrosis

Numerous hepatic and extra-hepatic mediators might play a role in the pathogenesis of fibrosis in NAFLD. Hepatic mediators are: (i) hepatocyte factors arising as a direct result of steatosis, hepatocyte injury and apoptosis including IR, reactive oxygen species and cytokines and (ii) Kupffer cell (KC), T cell, Hepatic stellate cells (HSC), and other inflammatory cell-derived factors released in response to hepatocyte injury and gut-derived bacterial products acting on toll-like pattern recognition receptors [148]. IR and hyperglycemia may induce fibrosis directly or via up-regulation of connective tissue growth-factor or the generation of advanced glycation end-products [149,150]. Both HSC and KCs engulf apoptotic cells to generate pro-fibrotic signals [151], with therapeutic prospects for anti-apoptotic agents [152]. CD4 Th2-cell production of IL-13 could contribute to fibrogenesis [153]. Hepatic progenitor cells can undergo an epithelial-mesenchymal transition, partly triggered by Shh, a hedgehog ligand [154]. This results in a pro-fibrogenic myofibroblast-like cell population, controlled by the hedgehog pathway [155] and amenable to pharmacological modulation [156]. With respect to signalling pathways involved in fibrogenesis, JNK1 signalling in KCs [157], NF-κB signalling in hepatocytes, KCs and HSC cells [158] and AMP-kinase mediated signalling in HSC [159] are all potential therapeutic targets.

Extra-hepatic mediators of fibrosis in NAFLD, include the gut, as a source of pro-inflammatory and pro-fibrogenic bacterial products including lipopolysaccharide, and visceral adipose tissue as a source of adipcytokines – many of which have direct pro-fibrogenic effects on HSC including leptin, renin-angiotensinogen and norepinephrine [160]. Equally important may be the reduced secretion of adiponectin in obesity, an anti-steatotic, anti-inflammatory and anti-fibrotic adipocytokine [161]. At least some of the anti-NASH effects of adiponectin may be exerted through the activation AMP-kinase which is also a target for metformin and glitazones [162].

Therapeutic management of NAFLD/NASH

Rationale for treatment choices

Therapy for NASH should prevent or reverse hepatic injury induced by lipotoxicity. One strategy is to correct IR and hyperinsulinemia and to reduce fat mass, in particular visceral adiposity. Weight loss and physical exercise, diet and lifestyle changes, insulin-sensitizing agents and anti-obesity surgery are all aimed at this objective. A second strategy is to prevent/reverse hepatic cellular damage induced by lipotoxicity. This can be achieved by inhibiting lipid peroxidation and oxidative stress, or through the use of anti-inflammatory, anti-apoptotic or other hepatoprotective agents. These two strategies may be at best combined, and future therapeutic research in NASH should focus on tailoring this dual approach to the individual patient. The treatment and monitoring of metabolic and cardiovascular comorbidities should be implemented alongside the hepatologic management.

Non-pharmacologic measures

Weight loss, physical exercise, reduction of sedentary lifestyle and dietary changes [163] should be implemented as first-line therapy, ideally on a long-term basis, in all patients with NAFLD/NASH, regardless of the severity of their liver disease. The best results are obtained with a multidisciplinary yet personalized approach [164]. The efficacy of these measures should be assessed after a 6-month period; if ineffective, additional therapeutic options such as pharmacologic therapy might then be considered. When earlier structured attempts had failed, additional therapeutic options might be considered earlier. In patients with steatosis alone or in young patients with NASH and no or minimal fibrosis, these measures are sufficient, if efficiently implemented and if clearly accompanied by a normalization of aminotransferases.

Weight loss and dietary measures

The minimal amount of weight loss for improving NASH has not been determined. A modest weight loss results in a significant reduction in liver fat despite minimal reduction in body fat [165,166]. A 5–10% weight loss can suffice for aminotransferase normalization [167,168]. Data from a small series has determined that a 9% weight loss improves steatosis significantly and inflammation marginally but not fibrosis [169]. At present, aiming for a weight loss of 7%, as proposed by International Societies on the basis of an extensive body of literature, appears to be a reasonable compromise in overweight and mildly obese patients.

The optimal type of weight-loss diet for NASH improvement is not known, as large controlled trials comparing different diets with histological end-points are lacking. A comparison of 4 weight-loss diets has shown that weight loss is similar regardless of macronutrient composition [170], so ultimately, any type of diet is likely to be beneficial as long as the patient adheres to it. However long-term data indicate that only 15% of participants lose more than 10% body weight, adherence drops after the first few months and most regain weight [170]. Behavioral therapy [164] could help and should be implemented whenever the required resources, which are considerable, are available. Regardless of weight loss, the consumption of certain dietary constituents appears to increase the risk of NASH/NAFLD and should be avoided. High fructose corn syrup contributes to IR and NAFLD [171], and therefore the consumption of soft drinks should be kept to a minimum as should the consumption of industrial *trans*-fats (present in many processed foods) which are also associated with the development of NAFLD and hepatic inflammation [172–174]. Finally there is a reduction of the omega-3/omega-6 polyunsaturated fatty acids ratio in the diet of NASH patients [175–177] and experimental and limited clinical data suggest that increasing this ratio by omega-3 dietary supplementation may lead to both metabolic and histological improvement [178–181].

There is no evidence that alcohol abstinence is beneficial for patients with NAFLD/NASH. In fact studies have shown an inverse association between modest wine drinking (less than a glass a day) and biochemical [182,183], ultrasonographic [184] or histologic [42] evidence of NAFLD/NASH with protective effects on diabetes, IR, and features of the metabolic syndrome [185–187].

Physical exercise and sedentary time

Patients with NAFLD engage in less than half the amount of exercise than age and sex-matched controls [112] and only 20–33% of them meet current recommendations for physical activity [188]. Reasons for not exercising include fatigue [189], reduced cardiorespiratory fitness [188,190], weight-related arthrosis, cardiovascular disease, and psychological factors [191]. Physical activity correlates inversely with intra-hepatic fat [192], increases insulin sensitivity [193], and reduces abdominal fat [194]. In obese individuals, short-term (4 weeks) aerobic exercise reduces hepatic fat and visceral adiposity even without a change in body weight or dietary intake [195]. Longer-term exercise (3 months) improves cardiorespiratory fitness, IR and liver enzymes independent of weight loss [196]. Physical activity targets derived from diabetes prevention trials and supported by International Societies could be applied to adult patients with NAFLD/NASH: at least 150 min per week of moderate-intensity physical activity (brisk walking) and at least 75 min per week of vigorous-intensity physical activity (jogging), in addition to muscle strengthening activities twice a week. However, individualized counseling is preferable and even limited physical activity is better than none, therefore any increase over baseline is preferable. Avoiding sedentary time outside the periods of physical exercise is equally important [197].

Bariatric surgery

In some bariatric surgery series massive weight loss has beneficial effects on steatohepatitis [198], and fibrosis [198–200], an overall improvement which was dependent on the correction of IR [201], although some controversy exists regarding the long-term histological benefits [202]. However, only highly selected morbidly obese patients are eligible for anti-obesity surgery. If otherwise indicated, anti-obesity surgery should be encouraged from the perspective of liver disease, especially in patients with advanced fibrotic NASH. We suggest that a systematic liver biopsy during surgery be performed because liver injury is asymptomatic even with advanced fibrosis or cirrhosis, steatohepatitis is frequent [23] and there is a high probability of reversal of liver lesions.

Pharmacologic therapy

Indications. Pharmacologic therapy directed at the correction of concurrent metabolic disorders (statins, antihypertensive agents, antidiabetic drugs, etc.) should be given as needed, as NAFLD does not increase hepatotoxicity or other side effects of these drugs. Pharmacologic therapy specifically aimed at improving the liver condition is indicated based on the potential for disease progression, the severity of fibrosis, and the potency of drugs to reverse or stop the progression of liver damage.

Steatosis. Pharmacologic therapy is not warranted for bland steatosis. Efforts should aim at preventing extra-hepatic complications of steatosis. Annual hepatic monitoring is warranted. Indeed, the initial liver biopsy could have missed lesions of steatohepatitis due to sampling error. Alternatively, additional metabolic risk factors can occur or worsen during follow-up (weight gain, occurrence of diabetes or dyslipidemia), which might increase the individual's risk of developing steatohepatitis. Optimal monitoring includes non-invasive follow-up of fibrosis (ideally serum markers together with elastometry), aminotransferases and markers of IR (fasting insulin and HOMA score) and could be done in a primary care setting. If there is fibrosis progression on repeated non-invasive assessment, worsening of metabolic risk factors and/or an otherwise unexplained increase in serum aminotransferases, a repeat liver biopsy, at least 5 years after the baseline biopsy, may be indicated.

Steatohepatitis (NASH). The aim of therapy is to prevent progression of liver fibrosis towards cirrhosis and/or decompensation of cirrhosis. Given the current understanding of natural history, NASH with no or minimal fibrosis (stages 0 or 1) does not require hepatic-targeted pharmacologic therapy. Liver-directed treatment could be indicated in NASH with intermediate fibrosis (Kleiner stage 2), high histological activity and risk factors for advanced fibrosis (age >50, diabetes, arterial hypertension or severe IR). NASH with bridging fibrosis (stage 3) and maybe, cirrhosis requires hepatic treatment. Annual non-invasive monitoring of fibrosis is warranted for all stages of disease whether or not patients are on hepatic treatment. Until non-invasive markers are fully validated, consideration should be given to interval repeat biopsies in patients with stages 2 or 3 disease to identify those patients who have progressed to cirrhosis who will require surveillance for the development of varices and HCC.

Specific liver-directed therapy

There are no approved medications for NASH, therefore the drugs discussed below should still be considered as experimental.

Glitazones are the only compounds having consistently shown some benefit in patients with NASH. Five randomized controlled trials are available [203–207], which are heterogenous for drugs and dosages, duration of treatment, categories of patients included and histological outcomes [208]. Almost all studies have shown a reduction in aminotransferase levels and steatosis and most an improvement in liver-cell injury and inflammation. None of them have shown a convincing benefit for fibrosis. Longer treatment, up to 3 years, does not confer additional histological benefit as most of the improvement occurs in the first year [209]. In a larger, multicentric US trial [207] pioglitazone significantly improved all histological aspects of liver injury (including resolution of steatohepatitis) more often than placebo, but not fibrosis. However, pioglitazone failed to achieve a predetermined primary composite end-point. Metformin had a beneficial effect on ALT in some [210,211] but not all [212] studies. Results on histology are conflicting [211,213] with controlled studies showing no benefit [214,215] possibly because the limited anti-steatogenic effect and small or no increase in adiponectin, at least in the short-term [216]. Other insulin-sensitizing agents such the weight-loss agent orlistat [169,217] seem ineffective. Ursodesoxycholic acid alone did not show a consistent biochemical and histological benefit at the dose of 13–15 mg/kg [218,219]; higher doses (30 mg/kg) induced a significant reduction in aminotransferase values, although it is uncertain whether this translates into histological improvement [220]. There are several small negative trials with vitamin E [211,221,222]; however, in a larger randomized trial 800 IU/day improved all histological lesions except for fibrosis [207]. In a small randomized trial, the combination of vitamin E and ursodesoxycholic acid (13–15 mg/kg) induced biochemical and histological improvement [223]. Other hepatoprotective agents such as betaine [224], pentoxifilline [225], or probucol were not convincingly effective in randomized trials. Only preliminary uncontrolled trial results are available for omega-3 polyunsaturated fatty acids and sartans. Given the current data and what has been discussed during this conference, a recommendation for pharmacologic therapy of NASH could be either a 1–2 year course of therapy with glitazones or vitamin E, preferably in association with high-dose ursodesoxycholic acid.

Guidelines for future therapeutic trials

Since there are no approved drugs for NASH development programs should be designed to meet relevant but also achievable end-points. For drug approval, histological end-points such as improvement in the NAS score and/or disappearance of steatohepatitis are relevant, as steatohepatitis and necroinflammatory lesions are clearly associated with progression of the disease [52]. These end-points are most likely achievable with 1 or 2 year trials with reasonably large sample sizes. Larger and longer trials would be necessary for documenting an impact on fibrosis or a reduction in incident cirrhosis or other hard end-points; therefore these end-points should be requested not for registration but for outcome trials, an important but not an initial step in the registration process. The type of patients to be included in registration trials are those with NASH regardless of fibrosis stage, as steatohepatitis is a potentially progressive condition in regards to liver fibrosis. A key aspect will be the thorough assessment for safety, as these drugs will be given to patients with numerous comorbidities and concomitant treatments. Therefore a long-term commitment from drug companies for carefully planned, post-marketing studies should be requested as part of the development program for all approved drugs.

Conclusion

NASH is an increasingly prevalent liver disease which increases overall and liver-related mortality. Steatosis might worsen insulin resistance and predict the development of metabolic or cardiovascular complications. Non-invasive diagnostic strategies should be developed particularly for the screening of the large number of individuals with metabolic risk factors. Collaboration between hepatologists and specialists in the endocrine, nutritional, and cardiology fields should be encouraged to optimize clinical management. Research efforts should be enhanced through a multinational European collaborative research program.

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Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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