
# Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Part 1: An Overview of the Diseases

Nonalcoholic fatty liver disease (NAFLD) is a widespread cause of chronic liver disease (CLD) and liver-related morbidity and mortality, and is the most common cause of CLD in North America.[1] United States guidelines for NAFLD management define this disease as: a) steatosis (accumulation of fat in the liver) with ≥5% fat infiltration in imaging or histology; and b) no alcohol-, drug-, or viral-induced steatosis.[2]

NAFLD has two principal clinical-histological phenotypes: (1) nonalcoholic fatty liver (NAFL); and (2) nonalcoholic steatohepatitis (NASH). Their differentiation is currently based on histologic findings present on liver biopsy. Classically, NAFLD is defined as the presence of fat on liver biopsy. The diagnosis of NASH includes the presence of fat on liver biopsy with the additional presence of inflammation, hepatocyte ballooning, and acidophilic degeneration. Fibrosis may or may not be present. NASH represents a growing challenge to public health, with links to increased mortality with an excess of cardiovascular-, liver-, and cancer-related deaths. Approximately one-third of patients diagnosed with NASH will ultimately die from liver disease.[3] NASH is currently the second most common indication for liver transplantation, and the second most common etiology for hepatocellular carcinoma.[4]

**Epidemiology**

As a generally accepted approximation, NAFLD affects somewhere in the order of 20–50% of adults in Western countries, and it is the most common liver disorder. Numerical differences in prevalence depend upon the specific population studied and the definition of NAFLD used.[5] NAFLD is more common in overweight individuals. It has been reported in 7% of normal-weight (lean) persons,[6] where it is more frequent in females with normal liver enzymes and younger ages. Their liver disease may be very progressive.[7] The prevalence of histologically-defined NAFLD in healthy potential living liver donors was found in two studies to be 20% and 51%, respectively.[8,9] In a study of nearly 400 middle-aged individuals, the ultrasonography-defined prevalence of NAFLD was 46%, while histologically-confirmed NASH was 12.2%.[10] In the Dallas Heart Study, the prevalence of NAFLD assessed by magnetic resonance spectroscopy (MRS) in the general population was 31%.[11] NAFLD incidence was found to be 20–86/1000 person-years based on elevated liver enzymes and/or on ultrasound, and 34/1000 per year by proton (1H)-MRS.[12] Estimates of the prevalence of NAFLD ranges from 6.3% to 33%, with a median of 20% in the general population. The estimated prevalence of NASH is lower, ranging from 3% to 5%.[5] The prevalence of NASH cirrhosis in the general population is not known.[2]

The global prevalence of NAFLD is estimated at around 25%, with the highest prevalence in the Middle East and South America, and the lowest in Africa.[13] The prevalence of NASH in Japan has been reported in 77.5% of patients with morbid obesity; 25% of the Japanese population is reported to have NAFLD, with 2% of those patients having NASH.[14,15] Metabolic comorbidities associated with NAFLD include obesity (51.3%), type 2 diabetes (22.5%), hyperlipidemia (69.2%), hypertension (39.3%), and metabolic syndrome (42.5%). US data indicate that 27–34% of the general population and 75–92% of morbidly obese individuals have NAFLD.[16] NAFLD prevalence in the US is estimated at 45% in Hispanic Americans, 33% in European-Americans, and 24% in African Americans.[17] The prevalence of NAFLD in Europe is also very high, with around one-quarter of the general population having NAFLD: rates range from 8% in Romania to 45% in Greece.[17] The prevalence of NAFLD in European patients with diabetes is estimated at 42.6%–69.5%, and, in those with metabolic syndrome, is estimated at 79%. Approximately one-third of patients with NAFLD ultimately develop NASH. The percentage of patients with NAFLD who develop NASH with subsequent cirrhosis is currently unknown.[18] There are no accurate data on the incidence of NAFLD in the US or Europe, partly due to the fact that this is usually a silent (symptom-free) disease that is discovered incidentally.[17]

The descriptive characteristics of individuals with NAFLD are comparable in North America and Europe, with an average age of 40–50 years in both regions. The majority (60–70%) of patients with NAFLD in the US are male; the distribution by sex appears similar in Europe, apart from specific countries such as Lithuania, where most NAFLD patients are reportedly female.[17] The majority (90%) of patients with NAFLD are overweight or obese. In addition, increasing age may be linked not only with development of NAFLD, but also with NASH.[17] NASH is more frequently seen in patients with metabolic syndrome.[19] Older patients with NAFLD have a higher likelihood of disease progression or mortality. Older age also increases the risk of developing related problems such as severe hepatic fibrosis, hepatocellular carcinoma, and type 2 diabetes mellitus.[20]

**Pathogenesis**

Ratzui and colleagues[21] provided a broad overview of the pathogenesis of NAFLD, considering perpetuating mechanisms, potential restorative mechanisms, and the impact of patient phenotype. The disease is usually initiated by either caloric overload (diet- and lifestyle-induced) or other potential mechanisms such as drugs. Following disease-initiating events, multiple cellular processes come into play. Some of these, such as progressive inflammation, cell death, and fibrogenesis, tend to perpetuate disease. Tissue repair pathways that tend to restore tissue and metabolic homeostasis also are activated. The balance between disease progression pathways and tissue healing pathways determines whether progression to cirrhosis occurs.

Many other authors have also provided information on this topic. The pathogenesis of NAFLD has been historically described as involving two 'hits' resulting in steatosis and leading to subsequent inflammation (steatohepatitis) and ultimately the development of cellular death and fibrosis. According to this traditional view, hepatic fat accumulation (i.e., pure fatty liver) is the 'first hit' and the prerequisite for hepatocyte injury to develop, whereas cytokines, adipokines, bacterial endotoxin, mitochondrial dysfunction, and/or endoplasmic reticulum stress represent the 'second hit' for the progression to NASH.[22] However, this theory has been challenged as knowledge of the interplay between insulin resistance, adipokines, adipose tissue inflammation, and other pathogenic factors has recently increased. It has been suggested that hepatic steatosis may represent several distinct injurious mechanisms rather than a true first hit.[23] For this reason, the two-hit theory for explaining the progression from NAFLD to NASH is now being modified by the 'multiple parallel hits' hypothesis.[24]

In the multiple-hit model, the first hit is insulin resistance and its associated metabolic disturbances. Hyperinsulinemia, caused by insulin resistance, results in an increased hepatic lipogenesis and impaired inhibition of adipose tissue lipolysis, resulting in an increased efflux of free fatty acids from the adipose tissue to the liver. After the initial hepatic infiltration with fat, the liver becomes extremely vulnerable to a series of hits that may follow, leading to hepatocyte injury and finally progressing from simple steatosis to NASH and fibrosis. Such multiple pathogenic factors can include upregulation of pro-fibrogenic pathways, increased oxidation, poorly regulated hepatocyte apoptosis, uncontrolled adopikines, and stellate cell activation.[24]

Pathways involved in the development of progressive disease with NASH and fibrosis include, but are not limited to, the following: cannabinoid receptor 1; microRNA; gut microbiome and intestinal bacterial endotoxins; adipose tissue peptides such as leptin, resistin, and adiponectin; and physiologic stress affecting the endoplasmic reticulum.[25] The accumulation of hepatic iron also plays a role by worsening oxidative stress and promoting liver fibrosis.[26]

**Diagnosis**

Currently, the definitive diagnosis of NAFL or NASH requires a liver biopsy, the only procedure that reliably differentiates NAFL from NASH despite limitations due to sampling variability.[27,28] However, liver biopsy is an invasive procedure that is associated with potential harm: it has a 0.5% complication rate and a 0.01% mortality rate.[29] Therefore, liver biopsy cannot be considered as a general screening procedure but should be reserved for selected patients that deserve a case by case assessment with discussion of risks and benefits.[30] Other diagnostic modalities are therefore of interest.

NAFL encompasses: a) steatosis alone; b) steatosis with lobular or portal inflammation, without ballooning; or c) steatosis with ballooning but without inflammation.[31,32] The diagnosis of NASH requires the presence of steatosis, ballooning, and lobular inflammation.[33–36]

Patients with NAFLD are usually asymptomatic. The most common presentations are incidental elevations in transaminases, detection of steatosis on imaging, or a new diagnosis of cirrhosis in the absence of alcohol use or chronic viral hepatitis.[5,37] Transaminase elevation and abnormal imaging are indirect biomarkers for the presence of NAFLD or NASH. However, transaminases can be normal in up to 50% of patients with NASH.[38] Radiological modalities for diagnosing NAFLD and NASH include variations of ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS).

Ultrasound evaluation of hepatic steatosis typically consists of a visual assessment of hepatic echogenicity with measurements of the difference between the liver and kidneys in echo amplitude and evaluation of echo penetration into the deep portions of the liver. Severity of steatosis is usually graded using a four-point scale: normal (grade 0), mild (grade 1), moderate (grade 2), and severe (grade 3).[39]

In patients without coexisting liver disease, ultrasound offers a fairly accurate diagnosis of moderate-to-severe hepatic steatosis with reported sensitivity ranging from 81.8% to 100.0% and specificity as high as 98%.[40] In contrast, ultrasound is not accurate in diagnosing hepatic steatosis when all degrees of steatosis are considered, with a reported sensitivity ranging from 53.3% to 66.6% and specificity ranging from 77.0% to 93.1%.[41,42] Because hepatic fibrosis may also increase hepatic echogenicity, the presence of underlying chronic liver disease may reduce the accuracy of ultrasound in the diagnosis of hepatic steatosis.[43]

CT evaluation of hepatic steatosis is based on the attenuation values of the liver parenchyma, evaluated as Hounsfield units (HU). As the attenuation value of fat (approximately 100 HU) is much lower than that of soft tissue, hepatic steatosis lowers the attenuation of liver parenchyma. Unenhanced CT scans are usually preferred to avoid the potential errors in contrast enhanced CT caused by variations in liver attenuation related to contrast injection methods and scan timing. Although the accuracy of CT in diagnosing hepatic steatosis has been found to vary, CT is accurate for the diagnosis of moderate-to-severe steatosis, but not as accurate for detecting mild steatosis. The threshold values of CT indices for the diagnosis of hepatic steatosis are also variable depending on the methods and populations used.[41,44] The potential hazard of ionising radiation makes CT unsuitable for use in children or for longitudinal monitoring of patients with NAFLD.

Unlike CT and ultrasound, which evaluate hepatic steatosis through proxy parameters (echogenicity and attenuation), MRI and MRS can more directly measure the quantity of hepatic fat. MRI and MRS both measure proton density fat fraction (PDFF), defined as the quantity of protons bound to fat, divided by the quantity of all protons in the liver, including those bound to fat and water.

Previous studies have compared the accuracies of MR techniques and other imaging modalities in the assessment of hepatic steatosis, with histologic grading as the reference standard. These studies consistently demonstrated that MRS and MRI outperform CT and ultrasound in the diagnosis and grading of hepatic steatosis. The MRI sensitivities and specificities in detecting histologic steatosis ≥5% were 76.7%–90.0% and 87.1%–91%, respectively, and the corresponding MRS performances were 80.0%-91.0% and 80.2%–87.0%, respectively.[39,41] From a practical viewpoint, MRI appears to have several advantages over MRS as the acquisition and analysis of MRS data requires expertise and is time-consuming.

More recently, multiparametric MRI has been introduced to the NASH diagnostic imaging options.[45] Unlike conventional MRI, multiparametric MRI uses a series of images to calculate quantitative parametric maps. Over the past few decades, many of these parameters have been shown to correlate with physiological measures such as iron, fibrosis, inflammation, and fat, offering the potential for noninvasive quantification of these properties. By combining multiple parametric maps, a comprehensive picture of liver tissue and disease can be generated. The calculated liver inflammation and fibrosis (LIF) score provides a total picture of ongoing inflammation and fibrosis throughout the liver: see Figure 1.

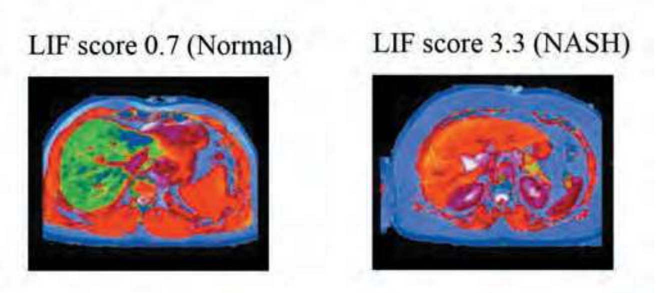


*Figure 1: Multiparametric MRI Imaging with Increasing LIF (Liver Inflammation and Fibrosis) Score and Image-Based Color Changes Consistent With NASH (Higher LIF Score Correlates With Color Change From Green to Red and is Consistent With Progressive NASH)*

Transient elastography (TE) uses low-frequency vibration acoustic waves, which are picked up by an ultrasound transducer. It measures the velocity of the sound wave as a marker of liver stiffness and reports it out as a numerical value. It is much more sensitive and specific for the later stages of fibrosis.[46] The TE device and procedure have also been improved to allow determination of hepatic steatosis with excellent sensitivity and specificity (between 78%–100%).[47] However, TE does not provide information on liver inflammation, a crucial component of the NASH diagnosis and clinical trial entry criteria.

Ultrasound elastography and MR elastography are emerging as promising methods to diagnose fibrosis associated with NASH. Ultrasound elastography and MR elastography evaluate liver stiffness by measuring the velocity of shear wave using ultrasound (ultrasound elastography) or MRI (MR elastography). Ultrasound elastography techniques demonstrate a stepwise increase in liver stiffness as the severity of histologic liver fibrosis increases. These can accurately differentiate advanced liver fibrosis from mild liver fibrosis, with sensitivities ranging from 88.9% to 100% and specificities ranging from 75.0% to 100%. However, the liver stiffness values do not correlate with the degree of hepatic steatosis or with hepatic inflammation. This indicates that ultrasound elastography can assess hepatic fibrosis associated with steatosis without being confounded by steatosis, but would not be able to assess hepatic inflammation.[48–50] MR elastography has shown that liver stiffness in patients with steatosis and lobular inflammation was significantly higher than in patients with steatosis only, and significantly lower than in patients with steatosis and fibrosis.[49]

**Serum Biomarkers**

There is no single reliable non-invasive serum biomarker that can verify the diagnosis of NAFLD or NASH. While prediction models for the diagnosis of NASH are reported in the literature, the majority of them comprise small sample size studies and lack adequate external validation. Serum biomarkers may be combined with other patient parameters to create scoring systems to predict the presence of NAFLD or NASH.

Three published models of scoring systems have relatively large sample sizes and some level of validation.[51–53] The NAFLD Fibrosis Score consists of six variables (age, body mass index, aspartate aminotransferase to alanine aminotransferase ratio, hyperglycemia, platelet count, and albumin level) and has been shown to reliably predict advanced fibrosis in a multi-centre trial. The Enhanced Liver Fibrosis (ELF) score has been compared to the NAFLD Fibrosis Score in predicting advanced fibrosis. The ELF is the Original European Liver Fibrosis (OELF) test with age excluded from the algorithm. The OELF test, a model consisting of age and three serum markers of matrix turnover, predicted advanced fibrosis in a variety of liver disorders. Another score of interest, the BARD score, is a weighted sum of three easily available variables, body mass index, aspartate aminotransferase to alanine aminotransferase ratio, and presence of type 2 diabetes.[54] There is ongoing evaluation of genetic pleomorphisms that may make patients more likely to have the diagnosis of NASH.

**Liver Biopsy**

Percutaneous liver biopsy is the gold standard for evaluating liver disease. It gives information on tissue architecture, steatosis, iron content, fibrosis, and cellular damage. However, due to the invasive nature inherent to biopsy, it is painful and carries the risk of bleeding and even mortality. Furthermore, a biopsy represents only 1/50,000$^{th}$ of the liver volume.[55,56]

The lack of a reliable and accepted non-invasive diagnostic test means that screening of the population for NASH has not been recommended at present. A better understanding of the natural history of NASH will be needed to evaluate the potential and cost-effectiveness of screening.[4]

The histological criteria for NASH diagnosis have been evolving since this was initially described.[57] The principal histological features of NASH include the presence of macro-vesicular fatty changes of hepatocytes, ballooning degeneration of hepatocytes, and a mixed lobular inflammation.[58] In some individuals, atypical features such as predominantly portal-based inflammation and fibrosis are present. This pattern has been observed more frequently in children and it is currently unknown whether it represents an early stage of steatohepatitis or a distinct condition.[59]

Two of various scoring systems are used most often for histologic diagnosis of NASH, as presented in Table 1. In an attempt to standardise the histological diagnostic criteria, the NIDDK-NASH Clinical Research Network (CRN) developed the NAFLD Activity Score (NAS).[33] This score consists of a sum of scores for steatosis, lobular inflammation, and hepatocellular ballooning. A NAS of 5 was almost universally associated with the diagnosis of NASH, and cases with a NAS of <3 were largely considered 'not NASH.' The NAS as a numeric value was not intended to replace a pathologist's diagnostic determination of steatohepatitis. The alternative is the steatosis, activity and fibrosis (SAF) score[34] with good validated reproducibility. The SAF score uses a simplified version of the Kleiner classification33 for fibrosis staging.[34] It measures the degree of steatosis (0–3), the activity (0–4) and the fibrosis (0–4). The activity score is a combination of inflammation (0–2) and hepatocyte ballooning (0–2). To date, the majority of clinical studies have used the NAS scoring system.[34]

**NAS: NAFLD Activity Score**
**SAF: Steatosis Activity Fibrosis Score**

| Scoring Systems | Steatosis Score | Lobular Inflammation Score | Hepatocyte Ballooning Score | Fibrosis Score |
|---|---|---|---|---|
| NAS | 0-3 | 0-3 | 0-2 | 0-4 |
| SAF | 0-3 | 0-2 | 0-2 | 0-4 |

The majority of liver biopsies are performed under ultrasound guidance. Biopsy size is an important but often unrecognised consideration in diagnostic accuracy.[27] A biopsy at least 1.6 cm in length with a 1.2–1.8 mm diameter, containing approximately 10 portal tracts, is considered adequate.[60]

The experienced pathologist plays a significant role in making the diagnosis of NAFLD/NASH, as in other types of liver diseases.[61] In a study of 32 adult liver biopsies for NASH read by nine pathologists, the inter-observer agreements on steatosis, ballooning, and fibrosis were reasonable. However there was considerable inter-observer variation for the location of steatosis and for assessing inflammation.[31] Similar observations were reported in a study of 21 liver biopsies read by eight experienced Japanese pathologists.[62] Interestingly, a 2016 survey reported that only 25% of practicing gastroenterologists used liver biopsy for NASH diagnosis, diverging from published practice guidelines.[63] This observation, however, must be seen in the light of the fact that there is no FDA- or other regulatory body-approved treatment for this disease.

**Paediatric Considerations**

In children, NASH displays features similar to those observed in adults, but the distribution of lesions is different. Portal inflammation is a frequent feature,[64] hepatocyte ballooning and Mallory-Denk bodies are rarely observed, and portal type chronic inflammation is more common.[33] Based on the distinctive histological pattern, a specific histological score (Paediatric NAFLD Histological Score, or PNHS) has been validated for better classification of children with/without NASH.[65]

Paediatric NAFLD/NASH liver biopsy interpretation requires special consideration. In paediatric NAFLD, the distribution of the fat and the prominence of portal inflammation and fibrosis may be different as compared with adults.[60] Two dominant subtypes of paediatric NAFLD have been described: Type 1, the least common, seen in Caucasian girls and with liver histology similar to adult NAFLD; and Type 2, the most common, seen usually in boys and characterised by either panacinar or periportal steatosis, portal-predominant inflammation, and portal-based fibrosis. Type 2 is also most commonly encountered in Asian, Hispanic, or Native American ethnic groups.[60]

**Current Management of NASH**

Current management of NASH is limited; there are no approved pharmacologic therapies for NASH. Various strategies are being examined to reduce liver diseases that are related to a sedentary lifestyle, overeating, and genetic factors.[3] Lifestyle intervention is the first-line therapy for patients with NASH, but few patients are able to lose 10% of their body weight, determined as the threshold for NASH improvement. Pragmatic approaches combining dietary restriction and a progressive increase in aerobic exercise/resistance training[66] are preferable and should be individually tailored. No data are available on their long-term effects on the natural history of NAFLD. However, epidemiological evidence suggests a relationship between lifestyle and NAFLD,[67] which makes lifestyle correction mandatory in all patients. In an uncontrolled, 12-month study with 261 paired liver biopsies, a modest lifestyle-induced weight loss was associated with NASH regression (25% of total cases) without worsening of fibrosis.[68] Bariatric surgery has been shown to be highly effective in severely obese patients with NASH, and may be proposed for patients who fulfill the validated criteria for this approach.[69]

**Looking Ahead to Part 2 of this Series: Clinical Trial Considerations**

The lack of approved pharmacologic therapies for NASH makes clear the pressing need for their development and commercialisation. Part 2 of this series, which will appear in the January 2018 issue of this journal, therefore addresses considerations for the future of clinical trials in this therapeutic area. Topics covered include trial design, biomarker and liver biopsy considerations, and efficacy outcome endpoints.

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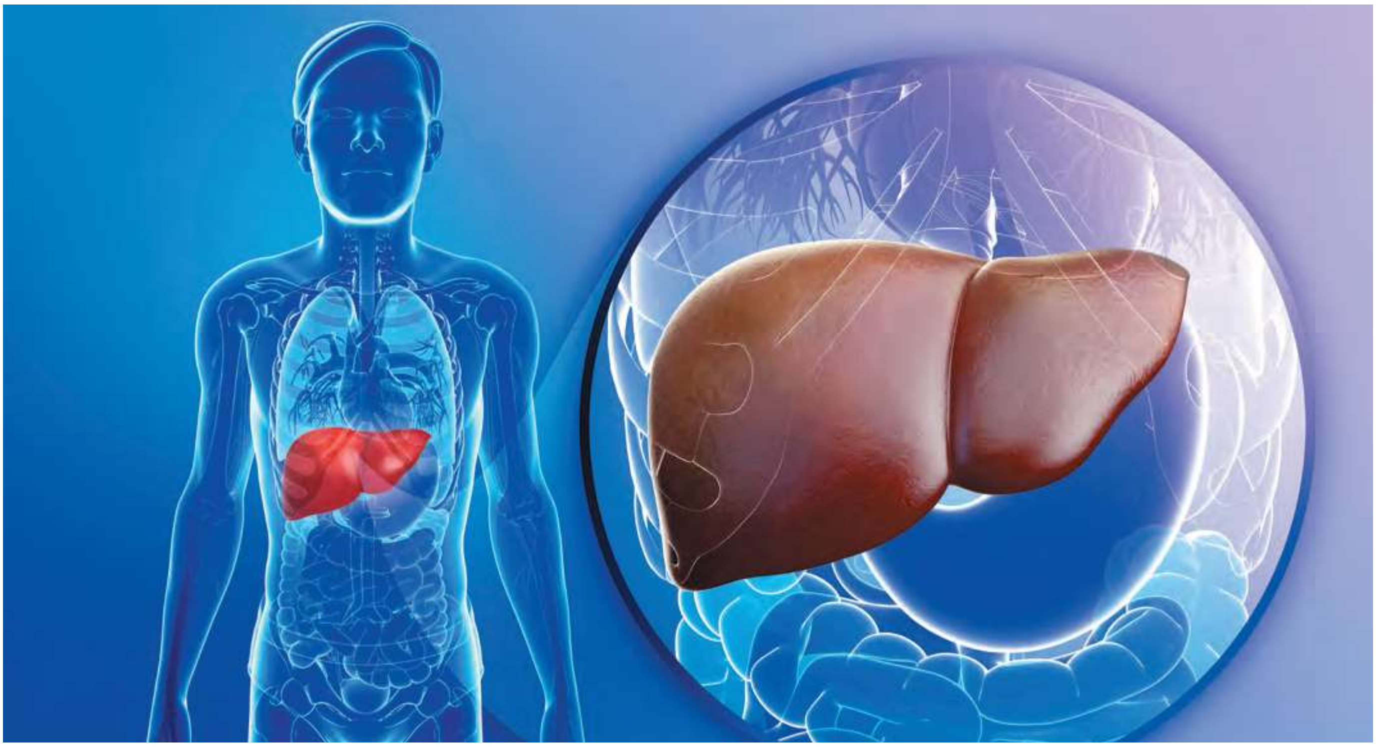

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