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# Hepatic steatosis and steatohepatitis: Are they really two distinct entities?

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## Abstract

Non-alcoholic fatty liver disease affects nearly 30% of Americans. A histopathological spectrum exists from simple steatosis to NASH which may progress to cirrhosis and HCC. NASH is currently the third most common indication for liver transplant with increasing incidence. Steatosis can be considered the hepatic manifestation of the metabolic syndrome as insulin resistance is a major risk factor for its development. While liver biopsy is the gold standard for diagnosis, non-invasive methods are currently being developed to appropriately determine who needs histologic evaluation. Management focuses on mitigation of risk factors, since targeted therapies to halt progression of fibrosis have not been validated. Simple steatosis does not affect overall survival, but NASH conveys increased mortality. Because of this, non-invasive strategies to diagnose patients and management algorithms are needed. This review supports the definitions of simple steatosis and NASH as two distinct entities based on pathophysiology, diagnosis, management, and prognosis.

### Keywords

Non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; NASH; NAFLD; steatohepatitis; steatosis; hepatitis; liver; cirrhosis; hepatocellular; transplant; obesity; insulin resistance; review

## Introduction

The clinical importance of non-alcoholic fatty liver disease (NAFLD) cannot be understated since population-based studies report evidence of hepatic steatosis in more than 30% of

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**Compliance with Ethics Guidelines**

**Conflict of Interest**

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Americans. It is the most common cause of chronic liver disease in Western countries [1]. NAFLD describes the accumulation of fat in hepatocytes exceeding 5% of the weight of the liver by biopsy or magnetic resonance spectroscopy (MRS) in a patient without a significant history of alcohol use. It encompasses a histopathological spectrum from bland steatosis to non-alcoholic steatohepatitis (NASH) which may progress to cirrhosis and hepatocellular carcinoma (HCC). The prevalence of NAFLD is expected to continue increasing as the obesity epidemic progresses [1, 2]. In an ethnically diverse population in a 2004 study, the prevalence of hepatic steatosis was found to be significantly higher in Hispanics (45%) compared to Caucasians (33%) and African-Americans (24%) [3]. A study seven years later of 400 patients found the rates of steatosis were 58%, 44%, and 35% respectively [4].

NASH is estimated to be present in 2 – 5% of the general population. It is defined on liver biopsy based on both the presence and pattern of distribution of liver lesions including steatosis, inflammation and hepatocyte ballooning with or without fibrosis. However, the prevalence of NASH in obese populations increases to 10 – 56% (median 33%) [5]. NASH cirrhosis is currently the third most common indication for liver transplantation in the U.S. but is expected to surpass alcoholic liver disease and hepatitis C virus (HCV) over the next decade [6]. NASH was originally described in 1980 by Ludwig and colleagues in a series of 20 patients showing steatohepatitis on biopsy without significant use of alcohol (daily intake of less than 20 g in females and 30 g in males). At the time, no cause or therapy was known [7]. After these observations, much has been learned about the pathogenesis and clinical significance of NAFLD, but a non-invasive diagnostic approach and effective management algorithms still remain elusive [8]. Questions exist about the likelihood of progression from simple steatosis to steatohepatitis. The purpose of this review is to discuss the similarities and differences between simple hepatic steatosis and steatohepatitis focusing on diagnosis, management, and prognosis.

## Risk factors

A correlation between NASH, truncal obesity, and diabetes mellitus type 2 has been recognized since its initial description. The presence of hypertension, dyslipidemia, and insulin resistance in an obese patient is characterized as the metabolic syndrome. Hepatic steatosis can be considered the hepatic manifestation of the metabolic syndrome. Insulin resistance due to genetic predisposition and a diet high in fat, carbohydrates, and calories is the key physiologic abnormality leading to the collection of fat, mostly triglycerides, in the liver [9, 10, 11]. A recent study illustrated this concept in overweight individuals when placed on a diet containing > 1000 kcal of simple carbohydrates a day for three weeks. It demonstrated an increase in liver fat of 27% by MRS compared to a total gain in body weight of 2% [12].

Clinicians must also be cautioned that a variety of medications, including total parenteral nutrition, amiodarone, tetracycline, and valproic acid, can lead to hepatic steatosis [13–16]. When evidence of the aforementioned risk factors is lacking, one should consider testing for celiac disease as a contributor. One study of 120 patients with NAFLD and body mass index (BMI) < 27 $kg/m^2$ found a 5.8% prevalence of celiac disease [17].

### Pathophysiology

Much has been discussed in the past decade about the pathogenesis of NASH and the role simple steatosis plays. Working from the original observation that peroxidation of liver fat was required to transition from simple steatosis to NASH, Day and James proposed a "two-hit" model in 1998 to explain how hepatic steatosis develops into NASH [18]. They proposed the first "hit" is fatty infiltration in the liver. The second "hit" is any source of free radicals that results in oxidative stress to the liver causing inflammation.

This prevailing theory was the accepted basis of pathogenesis for over a decade when Tilg and Moschen offered the "multiple parallel hits" hypothesis in 2010 as another mechanism to explain why many patients never progress past simple steatosis [19]. They described the initial "hit" as insulin resistance including its hepatic consequence of steatosis. The multiple "hits" that led to NASH were unregulated hepatic adipose tissue lipolysis, elevated endotoxins, changes in energy metabolism by the effects of one's microbiota, trans-fatty acids and fructose directly activating the aryl hydrocarbon receptor leading to inflammation, and an imbalance of adipocytokines from peripheral adipose tissue leading to the release of pro-inflammatory cytokines. The effects of these "hits" on the liver have been individually further described in other studies. [20–22].

Both of these models approach NAFLD as a progressive spectrum of disease from simple steatosis to NASH. However, a sufficient body of prospective clinical evidence showing biopsy transformation from simple steatosis to NASH has not been accumulated to clearly delineate this progression [23, 24]. Most evidence supports simple steatosis not progressing and having a benign course [25, 26]. In his excellent review, Y. Yilmaz thoroughly discusses an approach to NAFLD with simple steatosis and NASH as separate histological and pathophysiological diseases [27].

## Diagnosis

There are no discriminant findings on patient history or physical exam to rule out or diagnosis NAFLD. Simple steatosis and NASH are indistinguishable with these modalities as well. A common, non-specific finding in patients with NAFLD is fatigue that impairs their functionality [28]. Patients may also complain of right upper quadrant fullness or discomfort, while others are asymptomatic. Serum transaminases may be mildly to moderately elevated and the only laboratory abnormality found. The ratio of aspartate transaminase (AST) to alanine transaminase (ALT) is usually less than one, but may be higher if fibrosis is present [1]. Computed tomography (CT), MRS, or ultrasound can detect steatosis, but cannot reliably identify inflammation or stage fibrosis of the liver.

### Incidental finding

Hepatic steatosis may incidentally be found on thoracic or abdominal imaging in patients being evaluated for another reason. If the patient has abnormal liver enzymes or symptoms or signs suspicious of liver disease, then the most recent practice guidelines from the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology strongly recommend further investigation for the presence of NASH. When hepatic steatosis is found in patients with

normal liver enzymes and no symptoms or signs of liver disease, then it is recommended to evaluate the patient for metabolic risk factors of insulin resistance and dyslipidemia. These patients should be questioned about alcohol intake, but no independent investigation for NASH is warranted. Considering the gaps in knowledge regarding non-invasive diagnosis and treatment of NAFLD, it is not recommended to screen all patients for steatosis, even those at higher risk [29].

## Evaluation

When evaluating a patient for NAFLD, other causes of steatosis or chronic liver disease should be investigated. The patient should be tested for HCV, Wilson's disease, autoimmune liver disease, and hemochromatosis.

A simple calculation that suggests advanced liver fibrosis is the AST/ALT ratio. An elevation of the AST/ALT ratio was originally described as an indicator of advanced fibrosis in patients with HCV infection [Sheth 30]. It has since been used as a marker in other forms of liver disease. In NAFLD without advanced fibrosis, the AST/ALT ratio is usually less than one. However, these results are not consistently seen as fibrosis progresses to cirrhosis. In one study of 144 patients being evaluated by liver biopsy for NASH, 82% of patients with an AST/ALT ratio less than or equal to one had no fibrosis. But, 47% of patients with a ratio greater than one had advanced fibrosis [31]. While the AST/ALT ratio may be used to predict the presence of fibrosis, other diagnostic tools are needed to fully define the degree of fibrosis.

Liver biopsy is the gold standard to reliably distinguish NASH from simple steatosis without inflammation. Because of its invasive nature, clinical scoring methods, laboratory tests, and imaging techniques are being developed to help risk stratify a patient without a biopsy. Currently non-invasive scoring methods may assist a clinician in determining which patients are at high risk for NASH and should undergo a liver biopsy. The presence of metabolic syndrome can be used for initial risk stratification of patients who may need further evaluation for NAFLD by biopsy or non-invasive methods [29].

## Non-invasive diagnostic tools

One non-invasive scoring system to identify NAFLD patients with and without advanced fibrosis commonly used by general practitioners and gastroenterologists is the NAFLD Fibrosis Score (http://nafldscore.com/). It predicts the presence of advanced fibrosis based on six pieces of easily accessible data (age, impaired fasting glucose or diabetes, BMI, platelet count, albumin level, and AST/ALT ratio). Each of these was found to be independent predictors of advanced fibrosis [32]. A recent meta-analysis of 32 articles recommends that general practitioners use the NAFLD Fibrosis Score to identify patients at high risk for NASH and refer these patients to a gastroenterologist to evaluate for advanced fibrosis [33]. Also, practice guidelines strongly recommend its use in patients with metabolic syndrome [29]. The NAFLD fibrosis score may be used to predict the risk to develop liver-related complications and mortality in the long-term, as reported recently by three independent studies [34–36].

The Fibromax algorithm is another scoring method that has been tested in a family practice setting to identify patients who need to be further evaluated for NASH. One study found it predicted advanced fibrosis with a sensitivity of 50% and a specificity of 94.7% (likely because patients were excluded if they had risk factors for other causes of liver disease) [37]. This algorithm is not likely to gain wide spread usage because of the uncommon laboratory studies it requires (haptoglobin, gamma-glutamyl transpeptidase, apolipoprotein A1, and alpha 2-macroglobulin) and cost, and it has not been approved by the FDA for its use in the United States. The other components are serum insulin, transaminases, cholesterol, triglycerides, and bilirubin.

A serum marker for apoptosis of hepatocytes has recently been validated as a distinguishing marker between simple steatosis and NASH. Cytokeratin (CK) 18 fragments are detectable in the serum using monoclonal antibodies. A recent meta-analysis of 10 studies and 838 patients found pooled sensitivity of 0.83 (95% CI of 0.80 – 0.86) and specificity of 0.71 (95% CI of 0.66 – 0.76) for using CK-18 fragments to screen for NASH [38]. While this test has been validated as a useful screening tool for NASH, its lack of availability currently impacts its clinical use, and it has not been cleared by the FDA either.

Conventional magnetic resonance imaging (MRI), CT, and ultrasound can identify the presence of steatosis but are far less reliable at assessing fibrosis and inflammation. By identifying splenomegaly and reversal of portal flow, these modalities can diagnose portal hypertension, but they are of little use in delineating the earlier stages of fibrosis. Ultrasound-based transient elastography is an imaging technique using ultrasound that looks at liver stiffness to identify the presence and severity of fibrosis. Ultrasound based transient elastography is less accurate in patients with NAFLD as compared to other types of chronic liver disease given its limited success rate in patients with a higher body mass index and more severe steatosis [39, 40]. Ultrasound based transient elastography cannot distinguish between simple steatosis and NASH. MR-based elastography seems to provide a more accurate qanntification of liver fibrosis than the ultrasound-based technique and promises to be able to distinguish between simple steatosis and NASH with or without fibrosis [41, 42] but furthers studies are needed.

## Liver biopsy - the “gold standard”

Given the prevalence and lack of established therapies for NAFLD, there is hesitancy to perform liver biopsies on all patients with hepatic steatosis found on imaging. The most recent practice guidelines strongly recommend considering liver biopsy for diagnosis in patients with metabolic syndrome and a high NAFLD fibrosis score, as they are at higher risk for advanced fibrosis [29]. Developed in 2005, the NAFLD activity score (NAS) is a standardized approach to describe the presence and amount of steatosis, hepatocyte ballooning, lobular inflammation, and fibrosis on liver biopsy. This scoring system has been helpful in grading and staging these liver biopsy features [43], and their presence, severity and pattern of distribution is used to make the diagnosis of definitive NASH, no NASH, or borderline NASH [44].

Liver biopsy has its own inherent drawbacks that affect its reliability. In addition to the cost and morbidity associated with any invasive test, other limitations include sampling error and

variability of interpretation of the sample [45, 46]. Ratziu and colleagues demonstrated the extent of sampling error by comparing double biopsy samples from patients undergoing evaluation for NAFLD. While a small amount of variability is expected, most worrisome of their findings was the incongruity seen in 35% of patients who had bridging fibrosis on one sample and mild or no fibrosis on the other [47]. When considering that the "gold standard" for diagnosis of NAFLD has its own variation, there is concern that non-invasive tests for NAFLD are incorrectly assigned inaccuracy derived from their comparison to liver biopsy.

## Management

Lifestyle modifications to reduce obesity, insulin resistance, and hyperlipidemia are appropriate initial therapies for all patients with NAFLD. This is especially important in patients with evidence of NASH on biopsy because of the risk of progression to cirrhosis and HCC. Dietary changes (including avoidance of high-fructose corn syrup and trans fats) [48], the addition of physical exercise, and certain medications should all be considered when advising a patient with NAFLD. Multiple studies have revealed that weight loss from decreased calorie intake decreases steatosis and inflammation seen on MRS and NAS on repeat liver biopsy [12, 49–51]. In these studies, no repeat liver biopsies showed worsening steatosis or inflammation.

The implementation of regular physical activity is more beneficial to patients with NAFLD than strict dietary changes alone since these patients usually have a more sedentary lifestyle than non-fatty liver counterparts [52]. In addition to the metabolic syndrome, a sedentary lifestyle should be viewed as a risk factor for NASH. One study of 20 obese children with biopsy-proven NASH showed a statistically significant increase in sedentary scoring on a standardized survey compared to obese children without NASH and lean children. Also, less than 50% of children with NASH performed vigorous exercise defined as activities with metabolic equivalent (MET) values of ≥ 6 [53]. A retrospective analysis of 813 patients enrolled in the NASH Clinical Research Network concluded that while the duration of exercise recommended for prevention or treatment of NASH has not been established, the intensity of exercise may be a more important factor. This was demonstrated by a decreased odds ratio of having NASH (OR: 0.65 (0.43 – 0.98)) for those who met the US Department of Health and Human Services guidelines of ≥ 75 minutes of vigorous exercise a week [54].

### Risk of cardiovascular disease

Since cardiovascular (CV) disease is the most common cause of death in patients with NAFLD, it is important to take a "whole patient" approach to their care. A primary care provider must understand that patients with NAFLD have an increased incidence of CV disease and take appropriate preventative steps to control modifiable risk factors [29, 55–57]. The Framingham Risk Score can be used to calculate an individual patient's risk of CV disease as it has been shown to accurately convey the increased risk of patients with NAFLD [58]. To illustrate these risks, changes in cardiac structure and function have been detected on MRI in 19 patients with NAFLD without overt cardiac disease. Left ventricular wall thickening and concentric remodeling were seen in this group at a significantly higher rate than age, gender, and BMI matched healthy controls. These are considered early manifestations of increased myocardial strain [59].

## Medications

In addition to lifestyle modifications, practice guidelines consider statins safe and recommend their use in patients with NAFLD and dyslipidemia [29, 60]. A meta-analysis by Singh and colleagues reviewed ten studies including 1,459,417 patients with 4,298 cases of HCC. They found that statin use was associated with a reduced risk of HCC (adjusted OR: 0.63 (0.52 – 0.76)). Heterogeneity of their findings was attributed to study locations (Asian vs. Western populations) and different study designs [61, 62]. However, more randomized controlled trials (RCT) with histologic endpoints are needed to establish recommendations for use of statins specifically to treat NAFLD in patients without hyperlipidemia.

Given the benign course of simple steatosis, most efforts are focused on therapies for patients with NASH. Because of the known pathophysiologic mechanisms of NASH, insulin sensitizers and antioxidants have most thoroughly been investigated. Establishing histologic endpoints for therapies is inconsistent between studies given the invasive nature of repeat biopsies. Often markers such as steatosis on imaging or levels of transaminases are used as endpoints, but these can fluctuate over time even without treatment [33]. Pioglitazone is recommended for use in patients with NASH, but most studies have been done in patients without diabetes [63–65]. The PIVENS study, a multi-center RCT including 247 non-diabetic patients with NASH, compared pioglitazone, vitamin E, and placebo. Though the pioglitazone group did not meet the primary endpoint of improvement in NAS ≥ 2, significant changes in secondary endpoints were seen, including: improvement in insulin resistance and reduction in steatosis, inflammation, and levels of transaminases [65]. Long-term use of pioglitazone has been shown to convey a small increased risk of heart failure without increased mortality, and this medication has been associated to other adverse events such as osteoporosis and fractures as well as an increased risk for bladder cancer prompting the withdrawn of pioglitazone from the market in some countries. Thus, it seems the benefits of pioglitazone use do not outweigh these risks and it is not recommended for the treatment of NASH. Use of metformin and lifestyle modifications together for treatment of NASH has been shown in a recent meta-analysis to be no more effective than lifestyle modifications alone in improving liver histology or transaminases [33]. Although some patients may benefit from its lipid and glucose lowering effects, metformin is not recommended as targeted therapy for NASH [66].

Vitamin E has leads to promising improvement in NASH. The PIVENS study found that a significant number of patients met the primary endpoint mentioned above in the vitamin E arm compared to placebo (42% vs. 19%) [65]. Other studies have found improvements in NAS (most attributable to the change in hepatocyte ballooning) from vitamin E compared to placebo without significant change in transaminases or fibrosis [67, 68]. One caveat is that daily vitamin E has been shown to increase the risk of prostate cancer in healthy men (absolute increase of 1.6 per 1,000 person-years) [68]. The benefits of using vitamin E (800 IU/day) in patients with biopsy-proven NASH seem to outweigh the risks, and so it was considered first line therapy for non-diabetic patients with NASH by the practice guidelines [29]. However, the demonstration of increased overall long-term mortality with doses of vitamin as low as 400 IU/day found in meta-analyses [69] substantially decreases the

enthusiasm about using vitamin E in patients with NASH. Further, data regarding the efficacy of vitamin E in diabetic patients or patients with cirrhosis are lacking.

Other modalities are currently being studied for patients with NASH. Pentoxifylline, an anti-tumor necrosis factor alpha agent, may improve transaminases and fibrosis over 12 months [70]. But more studies are needed before recommendations can be made. Two promising medications are currently being evaluated for the treatment of NASH in large multicentric placebo-controlled trials; they include the obeticholic acid –a farnesoid X receptor agonist, and the GFT 505 –a peroxisome proliferator-activated receptor alpha and delta (PPARα/PPARδ) agonist.

Omega 3 fatty acids cannot be recommended as targeted therapy for NASH or NAFLD at this time, but are first line for treating hypertriglyceridemia [Chalasani 29]. The results of the recently completed placebo-controlled trial of EPA-E (omega 3 fatty acid) are eagerly waited. Blue green algae (by inhibition of lipid peroxidation), coffee (possibly by antioxidant effects), and probiotics (possibly by suppressing pathogenic bacterial growth) are being studied for their effects on NAFLD [71–74]. Heavy drinking should be discouraged in patients with NAFLD, but there may be some beneficial effects to modest alcohol intake (≤ 2 drinks a day) [75, 76]. Others are scrutinizing the current definition of NASH and argue that an artificial separation between alcoholic fatty liver disease and NASH is based more on the ability to identify risk factors than histopathological patterns [77]. Supporting this artificial distinction, Zhu and colleagues found that individuals with NASH had a significantly higher number of ethanol-producing gut bacteria and higher endogenous blood-ethanol levels than healthy controls [78].

## Prognosis

Simple steatosis and NASH are two entities most different in terms of prognosis. Simple steatosis does not affect mortality with similar long-term mortality figures as compared to the general population, while patients with NASH have decreased survival [79–81]. It is therefore imperative to develop noninvasive diagnostic techniques to identify the presence of necroinflammation, hepatocyte ballooning, and fibrosis to appropriately manage a patient's likelihood of progressing to cirrhosis and HCC. Given the prevalence of simple steatosis, the primary question regarding a patient's prognosis is, "does simple steatosis progress to NASH?". Currently, the majority of studies with coupled liver biopsies in patients with simple steatosis show no progression to fibrosis [25, 80, 82]. What has been shown is a slow, highly variable progression of fibrosis in patients with NASH. One study of 103 patients with coupled biopsies over a mean of 3.2 years showed increase, regression, and stabilization in fibrosis stage in 37%, 29%, and 34% of patients, respectively [25]. A study of 73 patients with NASH over two years showed a high degree of variation in ALT levels that did not correspond to changes in steatosis, inflammation, hepatocyte ballooning, or fibrosis stage. While ALT can be used as a marker to initially identify patients with disease activity, it lacks specificity to predict histologic changes. Even patients with normalization in ALT levels had increased fibrosis on repeat biopsy two years later [83]. Another study of 52 patients with paired liver biopsies over three years displayed highly variable results as well. Of the 13 patients with simple steatosis, follow up biopsies showed

simple steatosis (in 23% of patients), borderline NASH (defined as lobular inflammation but not as severe as NASH) (in 39%), NASH (in 23%), and normal liver histology (in 17%) [Wong 23]. Though the authors of this study reached the conclusion that simple steatosis may develop into NASH with fibrosis, we believe larger studies of patients with simple steatosis containing follow up biopsies are needed to clarify this progression. This variability may be due to sampling error, interpretation, or the current usage of NAS to predict fibrosis progression [45, 84, 85].

Söderberg and colleagues followed 118 patients with biopsy proven NAFLD over 28 years. They confirmed that patients with NASH had a higher risk of death than those with autoimmune liver disease but lower than those with alcoholic liver disease or chronic viral hepatitis. In patients with NASH, CV disease was the most common cause of death, followed by extrahepatic malignancy then HCC [79]. Another study of 247 patients with NAFLD over a mean of seven years found that their rates of liver-related complications and HCC were less than patients with HCV, however there was no significant difference in mortality rates between the groups. This may be attributable to the increased risk for CV disease in the NAFLD group [86].

### NASH and transplantation

NASH is the third most common indication for liver transplantation in the United States. The frequency of transplantation for HCV is gradually decreasing due to emerging therapies while the rates of transplantation for alcoholic liver disease have remained stable over the past decade [87]. The rates of transplantation for NASH increased from 3% in 2002 to 19% in 2011 [88]. If the rates continue to increase on this trajectory, then NASH may surpass HCV in the next decade to become the most common indication for liver transplantation [6, 88]. Clinicians should be aware that while outcomes for NASH-related transplantations are excellent, NAFLD recurs within the first five years in 39% of recipients. This did not affect survival, but given the correlation of NAFLD to the metabolic syndrome, control of risk factors for CV disease mentioned earlier must be repeatedly addressed in these patients [89].

## Conclusions

Over the past 30 years the rates of NAFLD worldwide have risen as more cultures adopt a western lifestyle and diet. As the prevalence of NAFLD continues to increase, further research is needed to develop non-invasive diagnostic approaches and management algorithms. From the perspectives of management and prognosis, simple steatosis and steatohepatitis are very distinct entities. A more sequential understanding of the development of fibrosis in NASH would provide targets for future therapies directed at halting the progression of fibrosis. To assist with future research of NAFLD, guidelines have been developed to standardize definitions, and to develop non-invasive markers of disease activity, and endpoints.

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