

Review Article

Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges

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The high prevalence of non-alcoholic fatty liver disease (NAFLD) has made the condition an important public health issue. Two clinical entities are manifestations of NAFLD, namely, non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). The former tends to be benign and non-progressive while the latter can progress to cirrhosis, which in rare cases gives rise to hepatocellular carcinoma. The diagnosis of NAFLD is based on: (i) a history of no or limited daily alcohol intake (<20 g for women and <30 g for men); (ii) presence of hepatic steatosis by imaging or by histology; and (iii) exclusion of other liver diseases. NAFL is defined histologically by the presence of bland, primarily macrovesicular, hepatocellular fatty change, while NASH features fatty change with inflammation and evidence of hepatocyte injury, such as ballooning degeneration. Presence of fibrosis is a sign of chronicity. Thus, the diagnosis of NAFL/NASH rests on clinicopathological criteria; it always requires

both clinical and biopsy-based information. NAFLD could be both the result and the cause of metabolic syndrome, with a vicious cycle operating between these conditions. Remaining challenges are: (i) the lack of a clear threshold alcohol intake for defining "non-alcoholic"; (ii) a lacking consensus for the classification of fatty liver disease; and (iii) absence of a histological definition of NASH, which currently remains the gold standard for the diagnosis. Further challenges include the overlap of the criteria for NAFLD and alcoholic liver disease as many obese individuals also consume considerable volumes of alcohol.

Key words: classification, clinical diagnosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, pathological diagnosis, steatohepatitis

INTRODUCTION

IN WESTERN COUNTRIES and many Asian countries, changes in diet and lifestyle have caused a dramatic increase in the prevalence of obesity and metabolic syndrome, which in turn has significantly increased the incidence of non-alcoholic fatty liver disease (NAFLD).¹⁻⁶ This condition is defined by the presence of hepatic steatosis in individuals with a history of only limited or no alcohol consumption. NAFLD could be both the result and the cause of metabolic syndrome, with a vicious cycle operating between these conditions.

Thus, NAFLD no longer is considered a primary liver disease, but rather a part of metabolic syndrome or insulin resistance and lifestyle-related diseases such as diabetes, dyslipidemia and hypertension.⁷⁻¹⁶ NAFLD occurs worldwide, with a prevalence ranging 10–50%. Age and sex differences in both the prevalence and severity of NAFLD reflect mainly the differences in the prevalence of obesity and metabolic syndrome in the general population.¹⁷⁻¹⁹ The prevalence of NAFLD also shows ethnic differences; it is higher in Hispanics than in white persons with European ancestry; it is low in African Americans.²⁰⁻²² Abdominal ultrasonography (US) during annual health screening of Japanese persons revealed evidence of NAFLD in 10–40% of adults.^{2,4,5,7,9,11,13} Such high prevalence made NAFLD an important public health issue.

Non-alcoholic fatty liver disease consists of two clinical entities, which are known as non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver

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(NAFL) or non-NASH. NAFL is generally a benign, non-progressive clinical entity, while NASH can progress to cirrhosis, which in rare cases gives rise to hepatocellular carcinoma (HCC).^{23–38} NASH is found in 10–20% of persons with NAFLD, and in Japan, the estimated prevalence of NASH is 1–8%. The diagnosis of NAFLD is based on the following three criteria: (i) a history of little or no alcohol consumption; (ii) presence of steatosis; and (iii) appropriate exclusion of other liver diseases.^{39–41} Because NASH is a clinicopathological entity, liver biopsy remains the gold standard for making a definitive diagnosis. However, there is no clear consensus regarding the threshold alcohol intake for defining “non-alcoholic” liver disease, the histological definition of NASH and the classification of fatty liver disease. In this review, we summarize the current concepts and problems related to these conditions.

CLASSIFICATION OF FATTY LIVER AND DIAGNOSTIC CRITERIA FOR ALCOHOLIC VERSUS NON-ALCOHOLIC LIVER DISEASE

TRADITIONALLY, FATTY LIVERS have been classified as alcoholic or non-alcoholic (Fig. 1). Based on epidemiological studies, alcoholic liver disease can occur when the daily alcohol intake exceeds 20 g in women and 30 g in men.^{42,43} For NAFLD, recent guidelines from the USA, Europe and Japan^{39–41,44} allow for ongoing or recent daily alcohol intake of less than 20 g for women and less than 30 g for men.

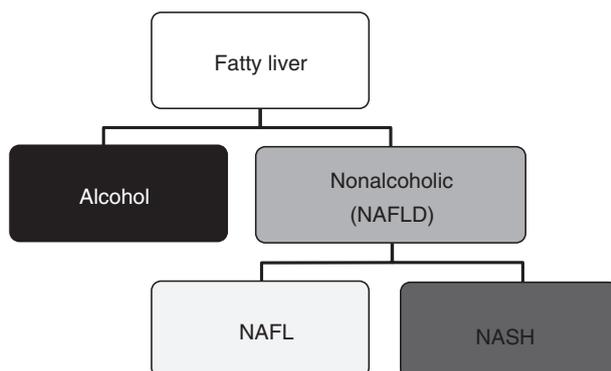


Figure 1 Classification of fatty liver. As shown, non-alcoholic fatty liver disease (NAFLD) may represent non-alcoholic fatty liver (NAFL) or non-alcoholic steatohepatitis (NASH), depending on the histological features.

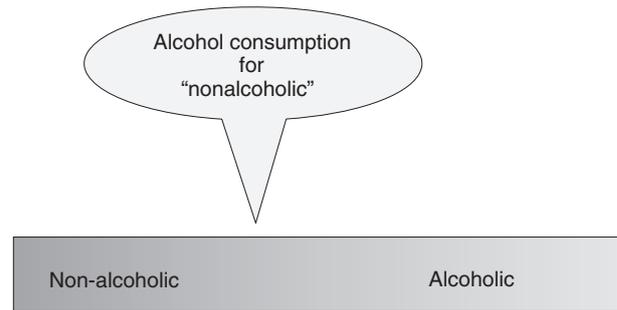


Figure 2 Schematic representation of how daily alcohol consumption merges from the clearly negligible range in cases considered “non-alcoholic” to cases that definitely fall into the “alcoholic” category. No consensus on a precise threshold between the two categories has been achieved and indeed may not exist. Based on current epidemiological studies, alcoholic liver disease can occur when daily alcohol consumption exceeds 20 g in women or 30 g in men.

DIFFICULTIES IN DISTINGUISHING ALCOHOLIC FROM NON-ALCOHOLIC LIVER DISEASE

DETERMINING ALCOHOL INTAKE in cases of suspected alcoholic liver diseases is a difficult task. Although total and daily ethanol intake are the most important risk factors for the development of alcoholic liver disease, the type of alcohol consumed and drinking patterns such as binge drinking also may have an influence.^{45–48} Also, individuals with alcohol dehydrogenase deficiency and female gender have increased susceptibility to alcoholic liver disease.^{48–51} Alcohol consumption may be difficult to interpret, for example, in an individual with a history of alcohol abuse for over 20 years who has stopped or reduced drinking within the past 3 years, or in persons with alcohol intakes of approximately 30 g/day who cannot give more precise details (Fig. 2).

Alcoholic liver disease, according to the Japanese criteria, can be diagnosed reliably if daily alcohol intake equals or exceeds 60 g.⁵² On this basis, the presence of fatty livers associated with daily alcohol consumption of 30 g to less than 60 g in men or 20 g to less than 60 g in women cannot be assigned with certainty to either NAFLD or alcoholic liver disease.

Many overweight or obese individuals consume alcohol excessively and thus may suffer from both alcoholism and metabolic syndrome. In northeast Germany, obese men with a daily alcohol intake more than 30 g accounted for 17.5% of the general population. Among men with hypercholesterolemia on US, or US-diagnosed

fatty liver, this proportion increased to 27.3%.⁵³ Surprisingly, modest alcohol intake appears to reduce the risk of NAFLD, possibly by reducing insulin resistance.⁵⁴⁻⁵⁶ On the other hand, in patients with cirrhosis, even minimal alcohol consumption appears to increase the risk of HCC. Ascha *et al.*⁵⁷ evaluated adult patients with cirrhosis secondary to chronic hepatitis C ($n = 315$) or NASH ($n = 195$), and the cumulative annual incidence of HCC was 2.6% and 4.0%, respectively. In both the chronic hepatitis C group and the NASH group, patients who never consumed alcohol were significantly less likely to develop HCC compared with those who reported any level of alcohol intake ($P < 0.001$).

To assess the influence of metabolic syndrome among patients with alcoholic liver disease who were hospitalized in our tertiary hospital, we studied 266 patients who had alcoholic liver disease, including 156 patients with cirrhosis.⁵⁸ Obesity was not common in these cases, compared with the general population. Rather, emaciation prevailed, particularly in women (Fig. 3). Our data suggest therefore that obesity plays no major role in the development of alcoholic liver disease. These findings suggest that complicated interactions appear to exist between alcohol consumption, obesity or metabolic factors, and the severity of the associated liver disease.

When NASH was first named by Ludwig *et al.* in 1980,²³ the authors mentioned that “the biopsy evidence of NASH sometimes caused clinicians to persevere unduly in their attempts to wrench from the patient an admission of excessive alcohol intake or to obtain a confirmation of such habits from relatives of the

patient”. Thus, the recognition of NASH immediately benefits the patients and their doctors by removing the stigma of alcoholism, with all its implications. At the time of the original studies, patient selection was based on the absence of appreciable alcohol consumption. Today, we need to deal with many patients who do not fall clearly into a “non-alcoholic” or “alcoholic” category. Therefore, the meaning of “non-alcoholic” is needed to be reevaluated.

ETIOLOGIC CLASSIFICATIONS

THE DISTINCTION BETWEEN primary and secondary NASH²³ appears obsolete. The term “primary NASH” was used if the cause of the liver disease was unknown, although the association with obesity or obesity-associated conditions was recognized. The term “secondary NASH” was used if patients had surgery for morbid obesity, evidence of an adverse drug effect or conditions such as abetalipoproteinemia or Wilson’s disease. In current medical practice, as discussed here, these conditions can and should be recognized by clinical history and laboratory studies. Unless otherwise specified, NASH today is considered the “primary NASH” in the early studies.

The term “non-alcoholic fatty liver” was first introduced in 1985 in a textbook of gastroenterology, edited by Bockus.^{59,60} In the chapter on NAFL, Schaffner introduced several putative clinical causes, including diabetes, obesity, jejunioileal bypass, kwashiorkor, microvesicular (fine droplet), steatosis in fatty liver of

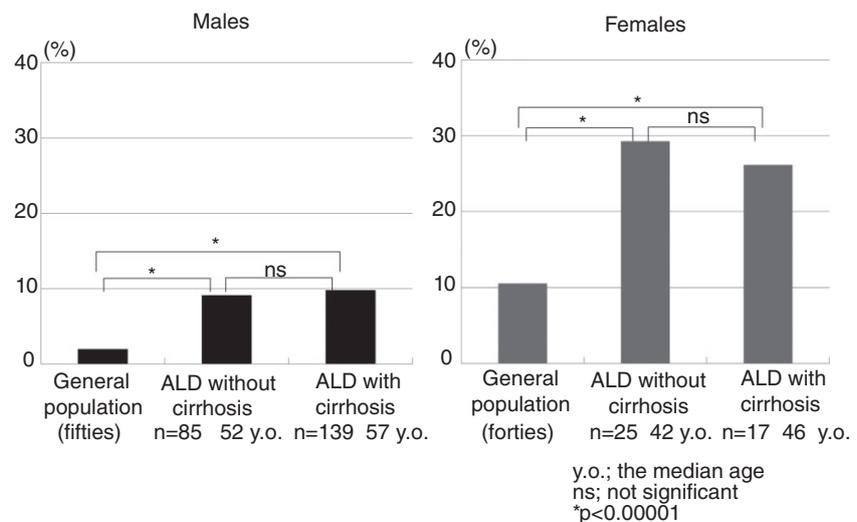


Figure 3 Prevalence of emaciation among Japanese males in their 50s and females in their 40s in the general population and among patients with alcoholic liver disease (ALD) with or without cirrhosis. Emaciation was more common in ALD patients, particularly in women, compared with the general population. * $P < 0.00001$. (Adapted from reference⁵⁸.)

pregnancy, tetracycline toxicity and Reye's syndrome. Fructosemia, carnitine deficiency, glutaric acidemia, urea cycle defects, cholesterol storage disease and drug-induced phospholipidosis also were mentioned. Again, as mentioned in the previous paragraph, such etiologic classifications are largely obsolete. Thus, patients with microvesicular steatosis have completely different clinical features, including acute severe liver failure and a high mortality rate. Microvesicular fatty change rarely is a biopsy diagnosis; its pathogenesis is not well known. Generally, the primary cause is mitochondrial injury that results in inhibition of the mitochondrial beta-oxidation of fatty acids.⁶¹⁻⁶³ Phospholipidosis also differs from NAFLD histologically, with deposition of sphingomyelin in hepatocytes and Kupffer cells.^{64,65}

In 2002, the American Gastroenterological Association (AGA) Technical Review on NAFLD stated that the pathogenesis of the condition, its natural history and its response to treatment all vary with the numerous causes of the clinicopathological findings.⁶⁶ It was proposed that complete diagnosis of fatty liver disease required determination of histology (steatosis and steatohepatitis, including the stage and grade) as well as a clinical etiology of the disease (including alcohol, insulin resistance, drugs, dyslipidemia, toxicity, weight loss and idiopathy).

Almost 10 years after the AGA Review, the American Association for the Study of Liver Diseases (AASLD), the American College of Gastroenterology and the AGA published new guidelines for the diagnosis and management of NAFLD.³⁹ They defined NAFLD as follows: (i) evidence of hepatic steatosis either by imaging or by histology; and (ii) absence of secondary causes of hepatic steatosis such as significant alcohol consumption, medications, Wilson's disease, starvation or parenteral nutrition, among others, and conditions associated with microvesicular steatosis. According to these guidelines, NAFLD consists only of fatty liver disease associated with obesity, type 2 diabetes mellitus, dyslipidemia and metabolic syndrome. In our opinion, these definitions are appropriate and very clear (Table 1). Thus, drug-induced fatty liver, which was included as a cause of NAFLD in the 2009 European Association for the Study of the Liver guidelines,⁴⁰ also has been dropped from the list of associated conditions.

For hepatologists and liver pathologists, these revised and simplified classifications are easy to understand, but colleagues in other fields such as cardiology or diabetology who frequently see patients with fatty livers, may not yet be familiar with the most recent etiologic classification of NAFLD.

Table 1 Fatty liver disease: histological and etiologic classification

Histological classification	
1) Steatosis	
2) Steatohepatitis	Grade: depends on degree of steatosis and necroinflammatory activity Stage: depends on degree of fibrosis
Clinical associations	
1) Excessive alcohol consumption	
2) Obesity	
3) Type 2 diabetes	
4) Dyslipidemia	
5) Metabolic syndrome	
6) Disorders of hormone	Polycystic ovary syndrome/hypothyroidism/ hypopituitarism/hypogonadism
7) Obstructive sleep apnea	
8) Pancreaticoduodenal resection	
9) Wilson's disease	
10) Lipid disorders (e.g. abetalipoproteinemia)	
11) Adverse drug reaction	
12) Exposure to toxic substances	
13) Starvation	
14) Total parenteral nutrition	
15) No known cause or association (idiopathic)	

Reproduced with modification.^{39,66}

HISTOLOGICAL DIAGNOSIS AND CLASSIFICATIONS OF NAFLD

NON-ALCOHOLIC FATTY LIVER disease (NAFL and NASH) has emerged as a distinct clinicopathological entity, but given the lack of surrogate markers for its morphology, biopsy still is considered the "gold standard" for a definitive diagnosis. The 2012 AASLD practice guidelines³⁹ defined NAFL (or non-NASH) by histological evidence of hepatic steatosis without signs of hepatocellular injury (no ballooning degeneration). NASH was defined by the presence of hepatic steatosis and inflammation, together with signs of hepatocyte injury (ballooning degeneration), with or without fibrosis. Cryptogenic cirrhosis was also included among the NAFLD-related diseases³⁹ because the histological characteristic of NASH may be lost with progression to end-stage cirrhosis, leaving cirrhosis without histological clues as to its etiology.^{67,68} Indeed, a large proportion of patients with cryptogenic cirrhosis probably had NASH in their past, as suggested by the high prevalence of obesity, metabolic syndrome and lifestyle-related diseases in this cohort.⁶⁹⁻⁷¹

Important pathological classifications of NAFLD/NASH were proposed by Matteoni, by Brunt, and the

NASH Clinical Research Network Pathology Committee; the authors of the NAFLD activity score (NAS).⁷²⁻⁷⁵

In the 1999 classification of Matteoni *et al.*,⁷² the authors distinguished between NASH and non-NASH. They divided 132 NAFLD patients into four categories: type 1 ($n = 49$) showed steatosis alone; type 2 ($n = 10$) steatosis with lobular inflammation; type 3 ($n = 19$) steatosis with ballooning degeneration; and type 4 ($n = 54$) was similar to type 3 but also featured Mallory–Denk bodies and/or fibrosis. Comparing the clinical characteristics and outcomes, the authors found cirrhosis and liver-related deaths almost exclusively among types 3 and 4. These findings prompted the authors to define histological types 1 and type 2 of NAFLD as “non-NASH”, while types 3 and type 4 were classified as NASH. This classification neither considered features such as the degree of steatosis or inflammation, nor the location of these changes (i.e. lobular or portal) or the severity of fibrosis. Hence, this classification provided a distinction between NASH and non-NASH and showed that ballooning degeneration could be interpreted more reliably than signs of non-specific inflammation. According to Mattaoni *et al.*,⁷² presence of steatosis with ballooning degeneration suffices for the diagnosis of NASH, while steatosis plus inflammatory changes alone are not diagnostic, even if both findings are severe. Although the number of type 2 or type 3 patients was small, the results strongly suggested a more benign clinical course of types 1 and 2 versus types 3 and 4. These findings were strengthened by a 2009 follow up of the same NAFLD patients.²⁸ Nevertheless, a few non-NASH patients also develop cirrhosis.

In 2009, a meta-analysis⁷⁶ of 10 studies,^{26,27,31,77-83} comprising 221 cases of NASH, indicated that patient age and the presence of inflammation at the initial biopsy independently predicted progression to advanced fibrosis. This emphasizes the importance of inflammatory changes.

When Matteoni’s classification was published, Brunt *et al.*^{73,74} proposed a semiquantitative grading and staging system for NASH. The authors graded steatosis, inflammation and ballooning degeneration, while staging was based on the degree fibrosis. Obviously, this classification is only applicable to NASH, and not to the entire spectrum of NAFLD. In 2005, the NASH Clinical Research Network Pathology Committee developed and validated a histological score for NAFLD, based on Brunt’s classification. Their NAS is a semiquantitative method of grading, designed to judge treatment responses or disease progression in clinical studies.⁷⁵ The NAS system addressed the full spectrum of NAFLD,

and is applicable to both adult and pediatric patients. The NAS represents the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3) and ballooning degeneration (0–2). Scores of 5 or more correlated well with the diagnosis of NASH, as confirmed by an experienced pathologist who studied the specimens independently. Scores of less than 3 correlated equally well with “not NASH”, while scores of 3 or 4 did not allow clear assignments to one or the other category. In regard to fibrosis, stage 1 means perisinusoidal fibrosis in zone 3 (perivenular area; delicate [1A] or dense [1B]), while presence of portal fibrosis without perisinusoidal fibrosis is defined as 1C (an atypical feature in pediatric and morbidly obese patients).⁸⁴⁻⁸⁷ Stage 2 is characterized by perisinusoidal and portal/periportal fibrosis. Stage 3 is defined as bridging fibrosis, while stage 4 reflects cirrhosis (Table 2). Although the authors reminded us that the NAS system is not intended for the diagnosis of NASH, it often is used as a surrogate method for this purpose. This latter practice cannot be recommended because a definite diagnosis of NASH does not always correspond with the NAS values, as shown in 2011 by a comparison of 976 adults in the NASH Clinical Research Network studies.⁸⁸

In meeting reports,^{39,44,75} fatty liver is defined as more than 5% macrovesicular steatosis by light microscopic examination of a hematoxylin–eosin-stained liver section (4–5- μ m thick) under a $\times 10$ objective lens. The minimal criteria for the diagnosis of steatohepatitis include the presence of more than 5% macrovesicular steatosis, inflammation, and liver cell ballooning, typically, in adults, with a predominantly centrilobular (zone 3) distribution.

In their original paper, Ludwig *et al.*²³ already reported that NASH mimics alcoholic hepatitis, based on the presence of striking fatty changes with evidence of

Table 2 Histological scoring system

NAFLD Activity Score (NAS)
Steatosis (0–3)
Lobular inflammation (0–3)
Ballooning (0–2)
Staging
Stage 1: zone 3 fibrosis, perisinusoidal fibrosis, portal/periportal fibrosis
Stage 2: perisinusoidal and portal/periportal fibrosis
Stage 3: bridging fibrosis
Stage 4: cirrhosis

Reference.⁷⁵

NAFLD, non-alcoholic fatty liver disease.

lobular hepatitis, focal necroses with mixed inflammatory infiltrates and Mallory bodies in most instances. Evidence of fibrosis was found in most specimens, and cirrhosis was diagnosed in 15% of their patients. This is of interest because currently the image of NASH has changed again. Quite a few pathologists cannot accept that fatty liver should be defined by more than 5% macrovesicular steatosis, or that the minimum criteria for the diagnosis of steatohepatitis require the presence of more than 5% macrovesicular steatosis, inflammation and liver cell ballooning of any severity. They diagnose NASH as they do with milder forms of alcoholic hepatitis. These controversies emphasize the difficulties that can be encountered in the diagnosis of NASH, particularly by the many pathologists who are not experts in non-neoplastic liver diseases.

The evolution of histological classifications and staging in NAFLD is reminiscent of the 1960s and 1970s, when chronic hepatitis was divided into chronic active hepatitis, chronic persistent hepatitis and chronic lobular hepatitis. These distinctions were thought to have some prognostic significance.⁸⁹ Notwithstanding the change of disease names such as chronic active hepatitis, most authors now simply focus on the stage of fibrosis, because this is the most important and reliable risk factor for the development of cirrhosis and HCC.⁹⁰⁻⁹²

In a study of the long-term liver-related mortality in NAFLD patients, based on pathological characteristics, Younossi *et al.*⁹³ followed for at least 5 years 209 patients with biopsy-proven NAFLD. Although on univariate analysis a number of pathological features (e.g. ballooning, portal inflammation and Mallory–Denk bodies) seemed to be associated with liver-related mortality, on multivariate analysis fibrosis was the only independent predictor of liver-related mortality. In NAFLD, fibrosis is also the most important histological feature.

As shown, no consensus exists at present about the best way to classify fatty liver disease. It remains doubtful that recommendations to change the nomenclature, such as using the term metabolic fatty liver or metabolic steatohepatitis would be helpful. As many patients with NAFLD/NASH are taken care of by cardiologists, diabetologists and other physicians, and not by hepatologists, frequent changes in terminology and classifications should be avoided.

In summary, the diagnosis of NASH remains based on two main criteria, namely the histological evidence of steatohepatitis and the absence of a clinical history of alcoholism. Thus, after pathologists diagnose

steatohepatitis, clinicians need to evaluate their patients to determine the underlying etiology. In all these instances, the initial diagnosis is based solely on descriptive histological findings: that is, such a diagnosis should not need to be changed because of subsequent clinical information. After this step, possible etiologies are considered, based on all available clinical information. In this manner, clinicians and pathologists can reliably formulate the final diagnosis. It is very important to keep in mind this process of diagnosing NASH.

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