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Histology of NAFLD and NASH in Adults and Children

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the liver disease associated with obesity, diabetes and the metabolic syndrome. Although steatosis is a key histological feature, liver biopsies of patients with NAFLD can show a wide range of findings, including portal and lobular inflammation, ballooning and apoptotic hepatocellular injury, Mallory-Denk bodies, megamitochondria and fibrosis. Nonalcoholic steatohepatitis (NASH) is a progressive subtype of NAFLD first defined by analogy to alcoholic hepatitis. The characteristic finding in NASH is ballooning hepatocellular injury, often accompanied by Mallory-Denk bodies and some degree of fibrosis. Young children may have an alternate pattern of progressive NAFLD characterized by a zone 1 distribution of steatosis, inflammation and fibrosis. Several grading and staging systems exist for use in natural history studies and clinical trials, but all require adequate biopsies to minimize errors due to under sampling. Some information about the short term natural history is available from paired biopsy studies. These studies have demonstrated that while NASH generally shows fibrosis progression over time, some patients will show regression of disease.

Keywords

Steatohepatitis; Liver Biopsy; Steatosis; Histology; Scoring; Staging

Introduction

Non-alcoholic fatty liver disease (NAFLD) and its more severe subtype, non-alcoholic steatohepatitis (NASH), are steatotic liver diseases that develop in the absence of significant alcohol use. NAFLD is most often associated with obesity, specifically central obesity, insulin resistance, and other insulin resistance syndromes, type II diabetes, and

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hyperlipidemia. Because of these associations, NAFLD is now recognized as the hepatic manifestation of the metabolic syndrome. It demonstrates a spectrum of liver disease characterized by the accumulation of lipid, mainly in the form of macrovesicular steatosis. The histological manifestations range from mild steatosis in only 5% of hepatocytes to more severe forms with both lobular and/or portal inflammation, ballooning hepatocyte injury, and fibrosis in varying patterns of distribution to the end stage of cirrhosis.¹ As with other chronic liver diseases, patients are at risk to develop hepatocellular or other primary liver carcinomas.²

There is a growing recognition of NAFLD as a serious disease. Even with the most conservative estimates NAFLD is very common in the US and probably is more common than hepatitis C and alcoholic liver disease and certainly significantly more prevalent in patients who are obese or diabetics. The prevalence of NASH is higher than previously estimated and is 5- to 6-fold higher than the estimated prevalence of chronic hepatitis C^{3,4}. The prevalence of NAFLD has been estimated to range from 2.8% to 46% throughout the world depending on the study population and the diagnostic tool used to verify NAFLD (eg, serology, imaging, liver biopsy).^{4,5}

Prevalence studies of NASH, the most clinically relevant subset of patients with NAFLD, depend on histologic evaluation. In an autopsy study from the late 1980s, the prevalence of steatohepatitis was significantly different between the markedly obese patients (18.5%) and the lean patients (2.7%).⁶ In Marchesini's study of well characterized NAFLD, 163 of 304 underwent liver biopsy and of those, 74% had NASH.⁷ More recently, Dr. Stephen Harrison and his team evaluated the prevalence of NAFLD and NASH in healthy US adults via ultrasound followed by liver biopsy. Of 328 patients who completed an ultrasound and questionnaire, 156 (48%) had at least 5% steatosis on ultrasound. And nearly 30% of patients with NAFLD had NASH on histologic review. Moreover, 9 (3%) of the NASH patients had significant fibrosis (more than stage 1). These patients were found to be more insulin resistant than those with mild fibrosis.⁴

Nonetheless, it remains challenging to estimate the true prevalence of a disease for which there is no serologic confirmatory tests and a liver biopsy is required for definite diagnosis.

Importance of liver biopsy in NAFLD

The presumptive diagnosis of NAFLD is often made in the setting of persistently elevated serum aminotransferases with a positive imaging study (often ultrasound), no history of significant alcohol use, and absence of other congenital or chronic liver diseases. However, 4 to 5% of patients with other chronic liver diseases may have NASH⁸ and autoantibodies may be present in significant titers in 20% of NAFLD patients.^{9,10} In order to correctly classify the liver disease and exclude other coincident liver diseases, a liver biopsy is required.^{1,11,12} While a liver biopsy can provide a wealth of information about the state of the liver, is it not practical to try to distinguish NAFLD from alcoholic liver disease (ALD) by histopathological examination only.¹³

A number of retrospective and prospective studies have suggested that simple steatosis without inflammation carries a benign prognosis while those with the lesions of NASH are

more likely to progress to advanced fibrosis and cirrhosis.^{14,15} NASH has also been the focus of clinical trials¹. It is therefore important to try to distinguish those with steatosis alone from those with NASH. Steatosis can be detected by imaging, using ultrasound, computed tomography or magnetic resonance imaging (MRI). Modern MRI methods can detect a fat fraction as low as 5%, making it useful for detecting clinically relevant steatosis.¹⁶ However, the distinction between steatosis and steatohepatitis cannot be made by current imaging methods nor can imaging detect the lobular arrangement of steatosis, which is useful to distinguish the pediatric pattern of NAFLD (described below).¹⁷ Non-invasive serological markers have had limited success at making the distinction between steatosis alone and NASH, although they may have value as screening tests to identify patients unlikely to have NASH.^{18,19} Use of liver enzyme tests alone can lead to overdiagnosis of NASH. Serum aminotransferases correlate poorly with histological activity. For example, in one early study NASH was incorrectly diagnosed by clinical tests alone prior to biopsy in 17% (4/24) of the cases studied by Sorbi et al.²⁰ In this study, liver biopsy changed the diagnosis in 4 patients with suspected NASH; 3 had normal histology and the fourth had primary sclerosing cholangitis (later confirmed by imaging). Normal levels of aminotransferases are also no guarantee that significant disease is absent. Some fraction of patients with metabolic syndrome who have features of NAFLD or NASH including fibrosis and cirrhosis will have aminotransferases within the normal range; thus these patients might not be identified by serologic screening tools.²⁰⁻²² A liver biopsy remains the only diagnostic tool that can distinguish steatosis from steatohepatitis, grade the severity of the liver disease and correctly classify cases with more than one diagnosis.

Pathology of NAFLD and NASH

The term NAFLD is typically used to refer to the whole constellation of nonalcoholic steatotic disease, while NASH represents a histologically specific pattern of liver disease. NASH may be identified in the presence of other chronic liver diseases⁸, while if only steatosis is present, it may not be related to the usual metabolic causes of NAFLD²³. NASH is the most common form of histologically progressed NAFLD and is almost always associated with some degree of fibrosis. It is unclear whether patients with steatosis but without NASH can progress to cirrhosis, although children with the pediatric pattern of NAFLD (discussed below) can develop advanced fibrosis without the typical features of NASH.

By definition, NAFLD has steatosis which is more than 5%.¹ The presence of less than 5% of steatosis is not regarded as clinically significant. This degree of steatosis is present in all cases of NASH with some exceptions, and these include cases with advanced fibrosis/cirrhosis, and rare cases of steatohepatitis which are due to drug exposure to amiodarone. Steatosis from patients with NASH is most often of the macrovesicular type with both large droplets of fat that push the nucleus aside and small droplets of fat that leave the nucleus in the center of the cell. However, in both alcoholic and nonalcoholic FLD, small patches of microvesicular steatosis may also be present (Figure 1). Diffuse microvesicular steatosis is not a feature of NAFLD but rather a manifestation of acute liver injury resulting from severe acute mitochondrial injury due to variety of etiologies, including alcohol, drugs (tetracycline, zidovudine, cocaine, valproic acid, aspirin (Reyes' syndrome)), acute fatty liver

of pregnancy, and rare metabolic disorders like carnitine deficiency. In most cases, the steatosis is mild to moderate in degree but may vary from minimal to marked.¹⁸ The distribution of steatosis within the hepatic acinus is characteristic; steatosis is most intense around the central veins (predominantly in zones 2 and 3) and the periportal areas are often spared in early disease.^{24,25}

The inflammatory changes in steatohepatitis are nonspecific and variable but are usually mild in comparison to chronic hepatitis or chronic cholestatic liver disease. Some degree of lobular inflammation is common in NAFLD (Figure 2). It is typically mild mixed inflammation but is nearly always present in an adequate needle liver biopsy. Clusters of mononuclear cells including T cells and macrophages infiltrate into hepatocyte plates. Microgranulomas are a common finding. Scattered apoptotic bodies/acidophil bodies are common but are not a conspicuous finding. Neutrophils may surround the ballooned hepatocytes, especially cells that have Mallory-Deck Bodies (MDBs) in a pattern called satellitosis. Severe, confluent inflammation or bridging necrosis is not a feature of steatohepatitis and should make one consider other diseases such as autoimmune hepatitis or drug injury. Mild portal inflammation and mild ductular reaction are more common in NASH than in cases of steatosis alone¹⁸. Interface hepatitis when present, it is focal and mild, but as with bridging necrosis, significant interface hepatitis should make one think of other chronic inflammatory liver diseases. Although, portal inflammation is not a diagnostic criterion of NAFLD, it is reported to be associated with clinical and histological features of advanced disease^{26,27}. As the amount of fibrosis increases in steatohepatitis, more inflammation is observed in portal areas or within fibrous bands, so that patients who are approaching cirrhosis will have more inflammation in their fibrous tracts more than patients who are early in their disease. However, when chronic portal inflammation is moderate or marked, one should consider excluding other chronic liver diseases. The presence of lymphoid aggregates, as in hepatitis C, numerous plasma cells, as in autoimmune hepatitis, and bile duct injury as in primary biliary cirrhosis is very unusual in NASH. Portal inflammation may persist or even increase after surgical or drug treatment for NASH, even as steatohepatitis resolves.²⁸⁻³⁰

The diagnostic feature of NASH is zone 3 hepatocellular injury. It takes the form of hepatocellular ballooning, with or without MDBs (Figure 3). The ballooned hepatocytes are generally larger than the surrounding hepatocytes and have distinctive rarified cytoplasm that is irregularly stranded and clumped. There is some variation in this histological appearance and cells with features that fall between classic ballooned hepatocytes and normal cells may be identified. Some investigators have used the loss of staining for keratin 8 and 18 as a useful marker of ballooning.³¹ Ballooning is not typically seen in young children, even those with fibrosis. The significance of ballooning was shown by the Cleveland Clinic study in which patients were subdivided in 4 types depending on histologic findings: types 1 and 2 had steatosis without or with inflammation, while types 3 and 4 there added ballooning and other features associated with the diagnosis of NASH.¹⁴ Cirrhosis developed more frequently in types 3 and 4. A follow-up study using the same patient cohort demonstrated that ballooning and MDBs were most closely associated with perivenular and perisinusoidal fibrosis in multivariable analysis of multiple histological features.³²

Mallory-Denk bodies are a common finding in both ALD and NASH although in NASH cases the MDBs are fewer in number and not as well formed as in typical cases of ALD. They may be seen in other diseases— particularly in severe cases of chronic cholestasis, in Wilson disease and as a reaction to certain drugs. In NASH, the MDBs are associated with the ballooned hepatocytes in zone 3. MDBs have been noted to be a marker of severity or a progressive disease in ASH and NASH. They result from active metabolic process and consist of aggregates of unfolded hyperphosphorylated keratins, heat shock proteins, ubiquitin and an early response gene product known as p62³³. MDBs can be highlighted using immunostains for ubiquitin or p62. When immunostaining is performed, they may also be seen in non-ballooned hepatocytes.

Fibrosis in both ALD and NASH begins in the peri-central region or acinar zone 3, this fibrosis can become quite very complex in the perisinusoidal spaces and eventually will progress to bridging fibrosis and cirrhosis (Figure 4). This pattern of fibrosis is highly characteristic of steatohepatitis and is different from other chronic liver disease patterns where the central veins are involved later in the disease process. Periportal fibrosis develops after the perisinusoidal fibrosis and is manifested as trapping of hepatocytes around the edge of the portal area and extension of short strands of collagen into the surrounding parenchyma. Fibrotic bridges may eventually form single bands between the portal areas and central veins without hepatocyte trapping or island formation. Masson trichrome or other connective tissue stains are essential to highlight the fibrosis and particularly useful in early fibrosis of steatohepatitis. NASH cirrhosis may retain all of the lesions of active steatohepatitis³⁴ but the steatosis may diminish below the 5% level. The active lesions of steatohepatitis may disappear in cirrhosis as well^{35,36} leading to a diagnosis of cryptogenic cirrhosis. It is likely that NASH is the underlying etiology of most cases of cryptogenic cirrhosis³⁷⁻³⁹.

There are a variety of other findings in NAFLD and NASH that have less clinical and diagnostic significance. Megamitochondria or giant mitochondria have been described in ALD and NAFLD but they are more characteristic of alcohol-related steatohepatitis. These are small intracytoplasmic discrete, eosinophilic globules, 3 to 10 um diameter, sometimes are rounded and less often they can be needlelike shaped. They are bright red on Masson trichrome stain and negative with PAS stain, distinguishing them from alpha-1-antitrypsin globules. On ultrastructural examination, they are dysmorphic and contain crystalline inclusions.⁴⁰ Lipogranulomas occur frequently in NASH, but are not helpful in the diagnosis. They may be associated with focal fibrosis and are frequently seen next to central veins or portal areas. This type of fibrosis, however, should not be confused with the perisinusoidal fibrosis of steatohepatitis, and there is no evidence to suggest that this localized change contributes to progressive fibrosis. Glycogenated nuclei are a common finding in NASH also, but may be seen in other metabolic diseases. The hepatocytes nuclei appear empty and the change is seen more often in periportal hepatocytes.

NAFLD and NASH in Children and Adolescents

With the increasing prevalence of obesity and diabetes in children and adolescents⁴¹ it is not surprising that NAFLD and NASH have been identified in this population. Both teenagers

and younger children may show the same patterns of NAFLD and NASH as adults, with zone 3 centered ballooning injury, inflammation, steatosis and perisinusoidal fibrosis. The disease tends to be milder in children and biopsy surveys show only a few percent with cirrhosis. In a large study of children and adolescents enrolled by the NASH Clinical Research Network (NASH CRN) only 1 of 177 patients had cirrhosis on biopsy.⁴² Another multicenter study in North America found no cases of cirrhosis among 108 children with NAFLD and NASH.⁴³ However, these same studies found 14% and 20% of their respective cohort had bridging fibrosis, confirming that advanced liver disease could be found in children.

The typical adult pattern of NASH is seen mainly in adolescents, while younger pre-adolescent children tend to have an alternate pattern of progressive liver disease. This pattern is characterized by steatosis that is most prominent in zone 1, forming a collar around the portal areas (Figure 5). The vacuoles of fat are largest in the hepatocytes nearest the portal areas and tend to decrease in diameter across the acinus to zone 3. Lobular and portal inflammation are present, but generally mild. Ballooned hepatocytes are difficult to identify, if present at all, and tend to be the same size as the surrounding non-ballooned hepatocytes. Mallory-Denk bodies are not seen in this pattern. The fibrosis pattern is also different, beginning around the portal areas, with hepatocyte trapping by collagen and extension of short septations into the surrounding parenchyma. When bridging fibrosis develops, the bridges connect the portal areas, leaving the central veins alone. Because of the zone 1 centric appearance of this pattern, the NASH CRN has termed this the zone 1 borderline pattern.^{23,42} Since this pattern of NAFLD usually lacks the characteristic features of NASH (namely typical balloon cells and Mallory-Denk bodies) there has been reluctance to use the diagnostic term steatohepatitis when describing this pattern. Table 1 contrasts the feature of classical NASH of adolescents and adults with the zone 1 borderline pattern.

Grading, Staging and Scoring in NAFLD and NASH

As NAFLD and NASH became subjects of serious clinical study, it became necessary for pathologists to develop systematic approaches for assessing the severity of the various lesions described above. However, unlike chronic hepatitis, in which inflammation and fibrosis are the only histological features of importance, NAFLD and NASH have other characteristics that need to be tracked. Steatosis and ballooning injury are the main additions to inflammation and fibrosis in NAFLD scoring systems, but other features, including acidophil bodies, Mallory-Denk bodies and the zonal location of steatosis are also important. Fibrosis staging is straightforward in chronic hepatitis, proceeding from none to portal/periportal to bridging to cirrhosis in a linear fashion. While fibrosis in NAFLD and NASH proceeds generally from none to perisinusoidal to periportal and perisinusoidal to bridging to cirrhosis, the first manifestation of fibrosis may be periportal, particularly in children. The relative weight of perisinusoidal vs periportal fibrosis may vary and none of the current staging systems address this adequately. The risk is that efforts to simplify staging and grading for analysis may obscure key elements of pathophysiology. Pathologists and clinicians studying NAFLD and NASH should keep in mind that scoring systems are limited in their ability to capture the rich complexity of pathology in this disease.

There are three scoring systems currently in regular use: the Brunt system⁴⁴, the NASH CRN system⁴⁵ and the SAF system⁴⁶. A comparison of grading and staging of individual histological features between these systems is shown in Table 2. The Brunt system was proposed primarily as a method for grading the severity of NASH and was not intended to be applied to cases that did not meet minimal criteria for steatohepatitis. The grading of steatohepatitis into mild, moderate and severe was based on an overall impression of the severity of steatosis, inflammation and ballooning, but most of the weight was given to ballooning. The NASH CRN system was developed specifically for use in clinical trials and natural history studies as a detailed method for tracking histological change. It was designed with both pediatric and adult patterns in mind and in order to cover the spectrum of changes from the mildest forms of steatosis alone to the most severe forms of steatohepatitis. A composite grade, the NAFLD Activity Score (NAS), was developed as a summary of the overall severity of injury. It is defined as the unweighted sum of the steatosis, lobular inflammation and ballooning scores and it varies from 0 to 8.⁴⁵ A diagnostic categorization of the changes as NAFLD but not NASH, zone 1 or zone 3 borderline patterns or definite steatohepatitis is made separately from the NAS. The SAF system is the most recent addition and has not been widely used in clinical trials yet. In this system, steatosis (S), activity (A) and fibrosis (F) are separately assessed. Activity is defined as the sum of the lobular inflammation and ballooning scores and varies from 0-4. The SAF defines the presence or absence of NASH based directly on the scores, setting it apart from the other scoring systems (Table 3). Because it has no accommodation for the zone 1 pediatric pattern it should not be used to classify NAFLD and NASH in children. Cases with clear ballooning and inflammation but only minimal steatosis (<5%) would not be classified as NASH in this system, which may cause interpretation problems in cases of cirrhosis or in evaluating a therapy that affect steatosis without changing the other features.

Sample and Observer Variability

Although, liver biopsy has remained as a core diagnostic tool and is regarded as the “gold standard” for evaluation of fatty liver diseases, there should be awareness of the sampling difficulties and problems of inter and intra-observer variation in grading and staging. The staging and grading systems described above were developed as semi-quantitative scoring systems for assessing the severity of injury. Interobserver variability studies have been performed for the NASH CRN and SAF system with adequate demonstration of reproducibility.⁴⁵⁻⁴⁷

In addition to issues of variation in interpretation, there are also potential pitfalls related to sampling.^{48,49} A needle biopsy of liver represents at best 1/50,000th of the entire liver and the severity of liver disease may not be evenly distributed throughout the liver. Bedossa and colleagues using computer modelling have shown that accurate grading and staging in chronic hepatitis requires a biopsy of at least 25mm, unfortunately an uncommon specimen in modern hepatological practice.⁴⁸ Recent studies have shown that this is also an important factor in evaluating non-alcoholic fatty liver disease. In NAFLD, differences in findings, and thus in “grade” and “stage”, have been demonstrated in recent studies^{50,51}. Several studies have shown that experienced liver pathologists can have good or excellent agreement on the lesions of interest in NAFLD: steatosis, ballooning, lobular inflammation and

fibrosis^{45,47,52}. It should be noted that studies that attempt to define sample variation generally do not account for the noise contributed by observer variation and so overestimate the potential to miss lesions based on sampling.

Histologically Based Natural History Studies

The natural history of NAFLD has been studied for decades—even before NASH was characterized as a finding in NAFLD. Prior to 1980, there was significant debate in the medical and pathological literature as to the significance of steatosis in non-alcoholic patients and its relationship to progressive fibrosis. Steatosis was a common finding, but evidence of chronic liver injury, namely advanced fibrosis and to a lesser extent inflammation, was often absent. Although alcohol-like steatohepatitic injury seemed to be present in certain specific cases such as following intestinal bypass,⁵³ there was reluctance to extrapolate the association of steatosis and cirrhosis to the general population^{54,55}.

The natural history of NAFLD has been explored in several types of studies. There have been large epidemiological studies with populations defined by liver enzyme elevation coupled with risk factors like obesity and diabetes. On a smaller scale there have been retrospective studies of patients biopsied in the past and shown to have NAFLD or NASH who have then been followed to clinically determined endpoints. The third type of study involves paired biopsies. These are short-term studies, with mean times between biopsies of 3 to 8 years. Information on disease progression can also be found in placebo arms of randomized clinical trials which use histology as an endpoint. Table 4 highlights a number of paired biopsy studies in NAFLD and NASH, while Table 5 shows studies with placebo groups that have paired biopsies.

A number of general conclusions about fibrosis progression can be drawn from these studies. First, there is clear evidence of disease fluctuation in terms of fibrosis stage over the short term. While some proportion of the apparent progression and regression is related to sample and observer variation, the percent of the population that changes (20-30%) is greater than observational noise would contribute. It is interesting to note that placebo groups often show histological improvement both in fibrosis and disease activity—possibly due to good medical care while patients are enrolled in a clinical trial. Second, while the percent of progression and regression is similar in the short-term placebo treated groups, in the longer term paired biopsies there is a clear bias toward progression. This finding was emphasized by two recent meta-analyses.^{56,57} Singh et al. found a mean fibrosis progression rate of 0.07 fibrosis stages/year in patients with fatty liver alone and 0.14 stages/year in patients with NASH. They identified an association between rapid progression and hypertension as well as a low AST to ALT ratio at baseline, but not with other clinical or histological parameters. Argo and coworkers found that fibrosis progression was associated with age and inflammation on the initial biopsy, but not with baseline obesity, diabetes or hypertension. One of the most significant findings of the more recent meta-analysis as well as two of the most recent longitudinal studies^{58,59} has been the observation that patients with NAFLD who do not have features of NASH are still at some risk of fibrosis progression and may develop NASH on subsequent biopsies. Because disease activity and stage apparently fluctuate over time, baseline risk factors for disease progression may be difficult to identify.

Indeed, while there is some suggestion that baseline obesity, diabetes and other features of the metabolic syndrome play a role in disease progression, the actual size of the risk remains unclear. Nevertheless, long-term follow-up studies emphasize the importance of fibrosis stage in prognosis⁶⁰. Since almost all cases of NASH have some degree of fibrosis it may be difficult to define the contribution of the severity of NASH (separate from stage) to long term prognosis.

While natural history studies of NAFLD and NASH have shed some light on disease progression over the short term, there remain unanswered questions:

- Is there a benign form of NAFLD or are all patients with NAFLD but not NASH at risk for progression?
- Can we create a reliable predictive model of progression, either from baseline clinical and histological findings or from a combination of baseline data with some longitudinal data?
- How much do we shift the natural history by providing patients with a good standard of care for obesity and diabetes, without offering a specific pharmacological intervention?
- How are changes in fibrosis tied to changes in other histological and clinical parameters?

Longitudinal observational studies of NAFLD and NASH coupled with periodic biopsies have the potential to address these questions in ways that studies using only non-invasive methods cannot.

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Key Points

- Nonalcoholic fatty liver disease (NAFLD) is a histologically complex disease with the potential to progress to cirrhosis
- Nonalcoholic steatohepatitis is a specific progressive subtype of NAFLD, characterized by ballooning hepatocellular injury in zone 3, steatosis, inflammation and often fibrosis
- NAFLD in pre-adolescent children has an acinar zone 1 pattern of steatosis, inflammation and fibrosis that usually lacks ballooning but may progress to advanced fibrosis
- Liver biopsy remains the gold standard for confirmation of the diagnosis and definition of the severity of histological findings
- Paired biopsy based natural history studies suggest that over the short term there may be fluctuation in disease severity and fibrosis

Summary

NAFLD and its progressive subtype of NASH are histologically complex diseases in which the pattern of changes drives the diagnostic classification. A liver biopsy remains the best method for characterizing the complexity of injury. Disease progression to cirrhosis clearly occurs in patients with NASH, but some patients with steatosis alone may be at risk to develop NASH. The lesions of NAFLD and NASH can be graded and staged in a fashion similar to other liver diseases and these methods can be used to study histological change in patients enrolled in natural history studies and clinical trials.

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Figure 1. Patterns of steatosis in NAFLD. A. Zone 3 center steatosis with sparing of periportal areas (40×). B. Zone 1 periportal steatosis with sparing of central vein region (100×). C. A patch of microvesicular steatosis in a case of NASH (600×).

Figure 2. Inflammatory infiltrates in NAFLD. A. Lobular inflammation in NASH. A microgranuloma is seen to the right of the small lymphocytic infiltrate. (600×). B. Portal inflammation is mild and may focally involve the limiting plate. (400×)

Figure 3. Ballooned hepatocytes and Mallory-Denk bodies in NASH. A. Ballooned hepatocytes near a central vein (400×). B. Ballooned hepatocytes with large Mallory-Denk body cytoplasmic inclusions (600×).

Figure 4.

Fibrosis progression in NASH. A. Early perisinusoidal fibrosis with delicate collagen strands between ballooned hepatocytes (600×). B. Advanced perisinusoidal fibrosis (200×). C. Bridging fibrosis with extensive networks of perisinusoidal fibrosis nearly encircling a regenerative nodule. (100×) D. Established cirrhosis (40×). (All Masson stains)

Figure 5.
Zone 1 Pattern of NAFLD. A. Steatosis surrounds and bridges between portal areas (40×).
B. Steatosis spares the central vein (top, center) but surrounds the portal area (100×). C.
Portal inflammation infiltrates adjacent parenchyma (200×). D. Periportal fibrosis is present
(Masson, 200×).

Table 1
Comparison of features of the pediatric zone 1 borderline pattern of NAFLD with typical steatohepatitis

Characteristic		
Population	Mainly pre-adolescent children	Older adolescent children and adults
Steatosis	Zone 1 to pan-acinar distribution	Zone 3 to pan-acinar distribution
Inflammation	Portal inflammation equal to or greater than lobular inflammation in early disease	Lobular inflammation generally more prominent than portal inflammation in early disease
Ballooning	Indistinct or absent	Present, usually in zone 3
Mallory-Denk Bodies	Absent	Present
Fibrosis	Periportal fibrosis to portal-portal bridging fibrosis	Perisinusoidal fibrosis in zone 3 to portal-central bridging fibrosis

Table 2
Comparison of the essential elements of NAFLD/NASH grading and staging systems

Numerical Grade or Stage	Brunt System	NASH CRN System	SAF System
Fibrosis Stage			
0	None	None	None
1	Zone 3 perisinusoidal fibrosis only	Perisinusoidal or periportal fibrosis; 3 substages defined	Perisinusoidal or periportal fibrosis;
2	Zone 3 perisinusoidal fibrosis and periportal fibrosis	Perisinusoidal and periportal fibrosis	Perisinusoidal and periportal fibrosis
3	Bridging fibrosis	Bridging fibrosis	Bridging fibrosis
4	Cirrhosis	Cirrhosis	Cirrhosis
Ballooning Grade			
0	None	None	Only normal hepatocytes
1	Mild, zone 3	Few	Few: Clusters of hepatocytes with rounded shape and reticulated cytoplasm
2	Prominent, zone 3	Many	Many: Enlarged hepatocytes (2× normal)
3	Marked, zone 3		
Lobular Inflammation Grade			
0	No foci	No foci	No foci
1	1-2 foci per 20× field	<2 foci per 20× field	<2 foci per 20× field
2	2-4 foci per 20× field	2-4 foci per 20× field	>2 foci per 20× field
3	>4 foci per 20× field	>4 foci per 20× field	
Portal Inflammation Grade			
0	None	None	
1	Mild	Mild	
2	Moderate	More than mild	
3	Severe		
Steatosis Grade			
0	None	<5%	<5%
1	33%	5% to 33%	5% to 33%
2	33% to 66%	33% to 67%	33% to 67%
3	>66%	>67%	>67%

Table 3
The SAF Diagnostic Algorithm for Defining NASH

Steatosis (S)	Lobular Inflammation	Ballooning	Total Activity Score (A)	Diagnosis
0	0, 1 or 2	0, 1 or 2	0 to 4	Not NAFLD
1, 2 or 3	0	0	0	NAFL
		1	1	NAFL
		2	2	NAFL
	1	0	1	NAFL
		1	2	NASH
		2	3	NASH
	2	0	2	NAFL
		1	3	NASH
		2	4	NASH

Table 4
Longitudinal studies of NAFLD and NASH using Paired Biopsies

Author (year)	N	Follow-up (years)	Fibrosis Progressed (N)	Fibrosis Regressed (N)	Factors
Lee (1989) ⁶¹	13	3.5	5	0	
Powell (1990) ⁶⁶	13	3.5	4	1	
Bacon (1994) ⁶²	2	5.5	1	0	
Teli (1995) ¹⁵	12		1	0	
Ratziu (2000) ⁶³	14	5.2	2	4	
Evans (2002) ⁶⁴	7	8.2	4	0	
Harrison (2003) ⁶⁵	22	5.4	9	4	AST
Fassio (2004) ⁶⁶	22	5.3	7	4	Obesity, BMI
Adams (2005) ⁶⁷	103	3.2	38	30	DM, BMI, Fibrosis stage
Hui (2005) ⁶⁸	17	6.1	9	0	None
Ekstedt (2006) ⁶⁹	68	13.8	29	11	None
Caldwell (2009) ⁷⁰	7	6	7		
Feldstein (2009) ⁷¹	5	3.4	4	0	
Sorrentino (2010) ⁷²	132	6.4	45	11	Fibronectin, HTN, HOMA-IR
Wong (2010) ⁷³	52	3	14	13	Increased BMI and Waist circumference
Hamaguchi (2010) ⁷⁴	39	2.4	11	12	Hemoglobin A1c
Pais (2011) ⁷⁵	6	5	6		
Pais (2013) ⁵⁹	70	3.7	20	20	Age, DM
McPherson (2015) ⁵⁸	108	6.6	45	20	DM at follow-up
Total	712		37%	18%	

Abbreviations: AST – aspartate aminotransferase; BMI – body mass index; HTN – hypertension; DM – diabetes mellitus; HOMA-IR – Homeostatic model assessment of insulin resistance

Table 5
Changes in fibrosis stage among placebo treated patients in randomized trials

Author (year)	N	Treatment time (weeks)	Fibrosis Progressed (N)	Fibrosis Regressed (N)	Mean Fibrosis Change	Mean NAS Change
Harrison (2003) ⁷⁶	22	26	3	9	-0.27	-0.1
Lindor (2004) ⁷⁷	57	104	12	12		
Belfort (2006) ⁷⁸	21	26		7	-0.15	-0.77
Dufour (2006) ⁷⁹	10	104			0.5	
Aithal (2008) ⁸⁰	30	52	6	6		
Raziu (2008) ⁸¹	31	52	6	5	-0.18	0
Abdelmalek (2009) ⁸²	28	52			0.4	-1
Haukeland (2009) ⁸³	24	26		4	0.08	-0.42
Nelson (2009) ⁸⁴	6	52			0	
Leuschner (2010) ⁸⁵	91	78			-0.08	-1.03
Promrat (2010) ⁸⁶	10	48			-0.3	-1.4
Sanyal (2010) ⁸⁷	72	96		22	-0.1	-0.5
Lavine (2011) ⁸⁸	58	96		19	-0.2	-0.7
Van Wagner (2011) ⁸⁹	7	52	3	0	0.4	-0.3
Zein (2011) ⁹⁰	26	52		4	0.4	-0.1
Wong (2013) ⁹¹	20	24		5	-0.1	-0.3
Sanyal (2014) ⁹²	55	52			0	-1
Takeshita (2014) ⁹³	12	24			0	-0.4
Neuschwander-Tetri (2015) ⁹⁴	109	72	31	19	0.1	-0.7
Summary	689		61/256 (24%)	112/477 (23%)		