

WJG 20<sup>th</sup> Anniversary Special Issues (12): Nonalcoholic fatty liver disease**Histopathology of nonalcoholic fatty liver disease/  
nonalcoholic steatohepatitis**

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**Abstract**

Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome, is the most common chronic liver disease, and the prevalence is rapidly increasing worldwide. Nonalcoholic steatohepatitis (NASH), the severe form of NAFLD, can progress to liver cirrhosis and hepatocellular carcinoma (HCC). Although noninvasive clinical scores and image-based diagnosis for NAFLD have improved, histopathological evaluation of biopsy specimens remains the gold standard for diagnosing NAFLD/NASH. Steatosis, lobular inflammation, and hepatocellular ballooning are all necessary components for the diagnosis of NASH; fibrosis is also typically observed. Other histopathological abnormalities commonly observed in NASH include hepatocellular glycogenated nuclei, lipogranulomas, and acidophil bodies. The characteristics of pediatric NAFLD/NASH differ from adult NAFLD/NASH. Specifically, steatosis and portal inflammation are more severe in pediatric NAFLD, while intralobular inflammation and perisinusoidal fibrosis are milder. Although interobserver agreement for evaluating the extent of steatosis and fibrosis is high, agreement is low for intralobular and portal inflammation. A recently reported histological variant of HCC, steatohepatitic HCC (SH-HCC), shows

features that resemble non-neoplastic steatohepatitis, and is thought to be strongly associated with underlying NASH. In this report, we review the histopathological features of NAFLD/NASH.

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**Key words:** Histopathology; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Pediatric; Interobserver variation

**Core tip:** Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome, is the most common chronic liver disease, with a rapidly increasing prevalence worldwide. Nonalcoholic steatohepatitis (NASH), the severe form of NAFLD, can progress to liver cirrhosis and hepatocellular carcinoma. Although noninvasive clinical scores and image-based diagnosis for NAFLD have improved, histopathological evaluation of biopsy specimens remains the gold standard for diagnosing NAFLD/NASH. In this report, we review the histopathological features of NAFLD/NASH.

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**INTRODUCTION**

Nonalcoholic fatty liver disease (NAFLD) is a condition in which excessive fat accumulates in the liver of a patient without a history of alcohol abuse. NAFLD is etiologically associated with systemic and hepatic insulin resistance, and is regarded as a hepatic manifestation of metabolic syndrome<sup>[1,2]</sup>. The number of patients with

**Table 1** Histopathological abnormalities in nonalcoholic steatohepatitis<sup>[7]</sup>

Histopathological abnormalities
Necessary components
Steatosis (macro > micro; accentuated in zone 3)
Lobular inflammation (mixed, mild; scattered polymorphonuclear leukocytes as well as mononuclear cells)
Hepatocellular ballooning (most apparent near steatotic liver cells, typically in zone 3)
Usually present; but not necessary for diagnosis
Perisinusoidal fibrosis (in zone 3)
Hepatocellular glycogenated nuclei (in zone 1)
Lipogranulomas (in the lobules; of varying size, but usually small)
Acidophil bodies or periodic acid-Schiff-stained Kupffer cells
Fat cysts
May be present but not necessary for diagnosis
Mallory-Denk bodies (in ballooned hepatocytes)
Iron deposition (in hepatocytes or sinusoidal lining cells)
Megamitochondria (in hepatocytes)

NAFLD is rapidly increasing worldwide, consistent with the increased prevalence of obesity. NAFLD is currently the most common chronic liver disease; the prevalence of NAFLD in the adult population of developed countries is approximately 30%<sup>[5]</sup>. NAFLD has also become a significant liver disease in children due to the increased prevalence of childhood obesity<sup>[4]</sup>.

NAFLD is classified into two categories: simple steatosis, in which only hepatocellular steatosis is observed, and nonalcoholic steatohepatitis (NASH), in which both necroinflammatory reactions and hepatocellular steatosis occur. Although simple steatosis generally has a benign clinical course, NASH, which can be present in one third of NAFLD cases, is a progressive disease that can advance to liver cirrhosis and hepatocellular carcinoma (HCC)<sup>[5,6]</sup>. Steatohepatic HCC (SH-HCC), a histological variant of HCC, is thought to be strongly associated with underlying NASH.

Various noninvasive clinical scores have been proposed to diagnose NASH and predict fibrosis, and imaging-based diagnosis of NAFLD has improved. However, histopathological evaluation of biopsy specimens remains the gold standard for diagnosing NAFLD/NASH. In this study, we review the histopathological findings of NAFLD/NASH.

## DIAGNOSIS OF NASH

When diagnosing NAFLD/NASH, other liver diseases such as alcoholic liver disease, drug-induced liver injury, Wilson's disease,  $\alpha$ 1-antitrypsin deficiency, and viral hepatitis have to be excluded clinically. In the summary report of the American Association for the Study of Liver Diseases (AASLD) Clinical Single Topic Conference on NASH held in 2002, the histopathological abnormalities of NASH were summarized<sup>[7]</sup> (Table 1). In this report, steatosis (macro > micro, accentuated in zone 3), lobular inflammation (mixed, mild), and hepatocellular ballooning (most apparent near steatotic liver cells, typically in

zone 3) were identified as the necessary components for the diagnosis of NASH. Fibrosis was not necessary for the diagnosis of NASH, although it is usually present<sup>[8]</sup>. Currently, most hepatopathologists diagnose NASH according to these criteria, although a complete consensus has not been reached.

When the histological patterns are not sufficient to make an unequivocal diagnosis of NASH but suggestive changes are evident, the term "borderline NASH" is used<sup>[9,10]</sup>. In a study on pediatric NASH, Patton *et al*<sup>[11]</sup> proposed a diagnostic categorization consisting of "not NASH", "borderline zone 3 NASH", "borderline zone 1 NASH", and "definite NASH". The diagnosis, borderline zone 3 NASH, is used for cases that have some, but not all, of the histological features of steatohepatitis; therefore, an unequivocal diagnosis cannot be made. Borderline zone 1 NASH corresponds to type 2 NASH, as defined by Schwimmer *et al*<sup>[12]</sup> (described later). The classification of "type 1 NASH" and "type 2 NASH" may be impractical, as discussed below, and the classification proposed by Patton *et al*<sup>[11]</sup> may be more practical.

## GRADING AND STAGING OF NASH

Brunt *et al*<sup>[13]</sup> classified the necroinflammatory grades of NASH as grade 1 (mild), grade 2 (moderate), and grade 3 (severe) based on the degree of hepatocellular steatosis, ballooning and disarray, and inflammation (intralobular and portal) (Table 2). Simultaneously, they proposed a scoring system for staging based on the location and extent of fibrosis: stage 1, zone 3 perisinusoidal fibrosis; stage 2, portal fibrosis with the abovementioned stage 1; stage 3, bridging fibrosis in addition to stage 2; and stage 4, cirrhosis (Table 2). The NASH Clinical Research Network (NASH CRN) later subclassified stage 1 into 3 categories: stage 1A, mild perisinusoidal fibrosis in zone 3; stage 1B, moderate perisinusoidal fibrosis in zone 3; and stage 1C, only portal/periportal fibrosis<sup>[14]</sup>. Stage 1C fibrosis is observed occasionally in children or severely obese patients.

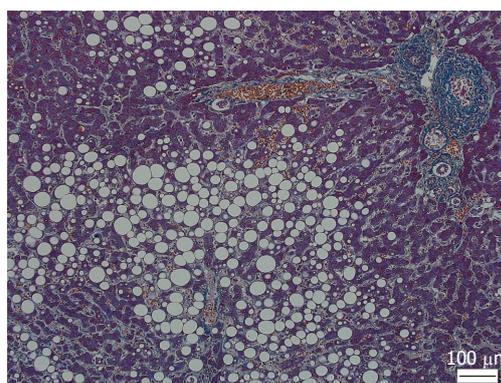
The NASH CRN designed the NAFLD activity score (NAS) for use in clinical research<sup>[14]</sup>. This score can be used for the full spectrum of NAFLD, including simple steatosis. The score is calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2), and ranges from 0 to 8 (Table 3). The main purpose of NAS is to evaluate histological changes over time, rather than serve as diagnostic criteria for NASH. However, some studies have used the threshold values of NAS, specifically  $NAS \geq 5$ , as a surrogate for the histological diagnosis of NASH, because  $NAS \geq 5$  has been reported to correlate with a diagnosis of NASH, and biopsies with scores of  $\leq 2$  were diagnosed as "not NASH"<sup>[14]</sup>. Recently, Brunt *et al*<sup>[15]</sup> reviewed biopsies from 976 adults in NASH CRN studies, and found that only 75% of biopsies with definite NASH had  $NAS \geq 5$ , whereas 28% of borderline NASH and 7% of "not NASH" biopsies had  $NAS \geq 5$ . In addition, 3%

**Table 2 Grading and staging system for nonalcoholic steatohepatitis<sup>[13]</sup>**

Grading and staging	
Grading	
Grade 1 (mild)	Steatosis: up to 66% Ballooning: occasional in zone 3 Intralobular inflammation: scattered polymorphs ± lymphocytes Portal inflammation: no or mild
Grade 2 (moderate)	Steatosis: any degree Ballooning: obvious, predominantly zone 3 Intralobular inflammation: polymorphs and chronic inflammation noted Portal inflammation: mild to moderate
Grade 3 (severe)	Steatosis: panacinar Ballooning: ballooning and disarray obvious, predominantly in zone 3 Intralobular inflammation: scattered polymorphs ± mild chronic inflammation Portal inflammation: mild or moderate
Staging	
Stage 1	Zone 3 perisinusoidal/pericellular fibrosis, focal or extensive
Stage 2	Zone 3 perisinusoidal/pericellular fibrosis + focal or extensive periportal fibrosis
Stage 3	Zone 3 perisinusoidal/pericellular fibrosis + portal fibrosis + bridging fibrosis
Stage 4	Cirrhosis

**Table 3 Components of nonalcoholic fatty liver disease activity score<sup>[14]</sup>**

Item	Definition	Score
Steatosis	< 5%	0
	5%-33%	1
	> 33%-66%	2
	> 66%	3
Lobular inflammation	No foci	0
	< 2 foci per 200 × field	1
	2-4 foci per 200 × field	2
	> 4 foci per 200 × field	3
Ballooning	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2

**Figure 1 Steatosis in nonalcoholic fatty liver disease.** Macrovesicular steatosis, predominantly distributed in zone 3, is observed (Masson trichrome staining).

of cases with  $NAS \geq 5$  were “not NASH”, and 29% of cases with  $NAS \leq 4$  were NASH. Therefore, the authors concluded that the diagnosis of definite NASH or the absence of NASH does not always correlate with the threshold values of NAS.

## HISTOPATHOLOGICAL FEATURES OF NAFLD/NASH

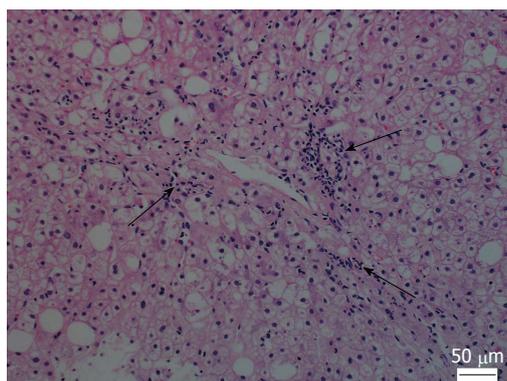
### Adult NAFLD/NASH

**Steatosis:** Hepatocellular steatosis is the hallmark of NAFLD, and steatosis in more than 5% of hepatocytes is required for the diagnosis of NAFLD<sup>[8,16,17]</sup>. Hepatocellular steatosis is classified into two types: macrovesicular and microvesicular. In macrovesicular steatosis, a single large fat droplet or smaller well-defined fat droplets occupy the cytoplasm of hepatocytes, pushing the nucleus to the periphery. In microvesicular steatosis, the cytoplasm of hepatocytes is filled with tiny lipid droplets, and the nucleus is located centrally in the cell. Steatosis

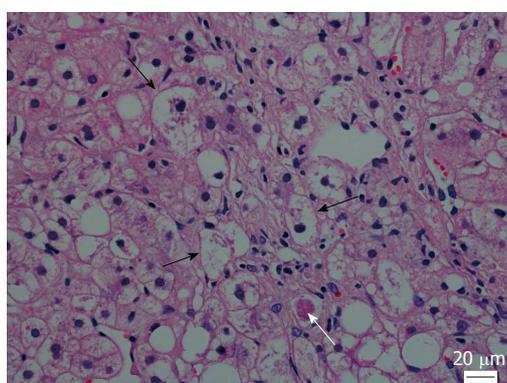
in NAFLD is usually macrovesicular; however, microvesicular steatosis may also be present. It was reported that microvesicular steatosis is present in approximately 10% of biopsies from patients with NAFLD<sup>[18]</sup>. Steatosis in NAFLD usually begins in zone 3 (Figure 1), although panacinar steatosis may also be seen with severe steatosis. An increasing severity of steatosis was reported to be positively associated with lobular inflammation, zone 3 fibrosis, and definite steatohepatitis<sup>[19]</sup>. In the same study, panacinar steatosis was more often associated with ballooning, Mallory-Denk bodies (MDBs), and advanced fibrosis compared with zone 3 steatosis.

### Lobular and portal inflammation

Intralobular inflammation is also present in NASH (Figure 2); it is usually mild, and consists of a mixed inflammatory cell infiltrate (lymphocytes, neutrophils, eosinophils, and Kupffer cells). Polymorphs sometimes surround ballooned hepatocytes (that typically contain a MDB); this lesion is referred to as “satellitosis”. Although satellitosis



**Figure 2** Lobular inflammation in nonalcoholic steatohepatitis. Necroinflammatory foci (arrows) are scattered in the hepatic lobule (hematoxylin and eosin staining).



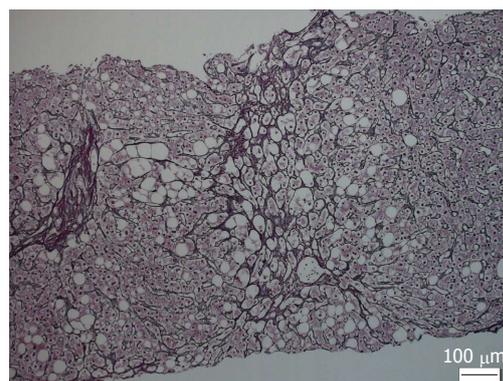
**Figure 3** Ballooning and Mallory-Denk bodies in nonalcoholic steatohepatitis. Ballooned hepatocytes are recognized as swollen hepatocytes with rarefied cytoplasm (black arrows). Mallory-Denk bodies are eosinophilic irregular-shaped aggregates in the cytoplasm of hepatocytes (white arrow) (hematoxylin and eosin staining).

can be occasionally seen in NASH, it is more common in alcoholic hepatitis<sup>[20]</sup>. Scattered lobular microgranulomas (sinusoidal Kupffer cell aggregates) and lipogranulomas (consisting of fat droplets as well as admixtures of inflammatory cells and collagen) are also often observed in NASH.

Portal inflammation in NAFLD/NASH is usually absent or mild, and consists mainly of lymphocytes. When portal inflammation is disproportionately severe, the possibility of concurrence of other liver diseases (such as hepatitis C and autoimmune hepatitis) should be considered. In the convalescent stage after treatment for NAFLD/NASH, portal chronic inflammation may increase, or not decrease, relative to the lobular features<sup>[21]</sup>. Chronic portal inflammation (greater than mild) has been associated with the amount and location of steatosis, ballooning, and advanced fibrosis<sup>[22,23]</sup>. Therefore, greater than mild chronic portal inflammation in untreated NAFLD could be considered a marker of advanced disease.

### Hepatocellular ballooning

Hepatocellular ballooning is characterized as swollen he-



**Figure 4** Fibrosis in nonalcoholic steatohepatitis. The characteristic pattern of fibrosis in nonalcoholic steatohepatitis is perisinusoidal/pericellular (chicken wire) fibrosis, which usually begins in zone 3 (reticulin staining).

patocytes with rarefied cytoplasm, and reflects hepatocellular injury (Figure 3). Fat droplets and/or MDBs may be observed in ballooned hepatocytes. Hepatocellular ballooning is believed to result from alteration of the intermediate filament cytoskeleton. In ballooned hepatocytes, the two hepatocyte keratins cytokeratins 8 and 18 are disrupted and no longer present throughout the cytoplasm; instead, they are dispersed to the periphery<sup>[24]</sup>. Although recognition of ballooned hepatocytes may be difficult in slides stained with hematoxylin and eosin (H and E), the loss of cytokeratin 8/18 immunostaining can serve as an objective marker of ballooned hepatocytes<sup>[25]</sup>.

### Fibrosis

The characteristic pattern of fibrosis in NASH is perisinusoidal/pericellular (chicken wire) fibrosis, which typically begins in zone 3. Masson trichrome or reticulin staining can be useful to evaluate fibrosis (Figure 4). Recently, very fine non-zonal sinusoidal fibrosis was reported, particularly in adult NAFLD patients post-intervention<sup>[26]</sup>. Fibrosis in NAFLD is usually observed with an active necroinflammatory reaction; however, fibrosis without active lesions can also occur, and prior episodes of steatohepatitis are suggested in such cases<sup>[27]</sup>. As NASH progresses, portal/periportal fibrosis, bridging fibrosis, and liver cirrhosis may occur. In a meta-analysis of ten longitudinal histological studies, older age and parenchymal or portal inflammation on the initial biopsy were independent predictors of progression to advanced fibrosis in NASH<sup>[28]</sup>. During advanced fibrosis or cirrhosis, steatosis and necroinflammatory reactions may disappear, and this status is known as burn-out NASH<sup>[27,29]</sup>. Patients with these symptoms are diagnosed with cryptogenic cirrhosis and NAFLD/NASH is estimated to be a leading cause of cryptogenic cirrhosis<sup>[30-33]</sup>. NASH-related cirrhosis is most commonly macronodular or mixed<sup>[18,34]</sup>.

### Glycogenated nuclei

Glycogenated nuclei are vacuolated nuclei observed usually in periportal hepatocytes, and commonly occur in NAFLD. The presence of glycogenated nuclei is useful

for discriminating between NASH and alcoholic steatohepatitis (ASH), as they are rarely observed in ASH<sup>[35]</sup>.

### **Apoptotic hepatocytes (acidophil bodies)**

Apoptotic hepatocytes (acidophil bodies) are observed often in NASH<sup>[36]</sup>, and are deeply eosinophilic rounded bodies, with or without hyperchromatic nuclear fragments. These structures are most commonly observed in sinusoids.

### **MDBs**

MDBs are eosinophilic irregular-shaped aggregates found in the cytoplasm of hepatocytes (Figure 3). These structures are usually observed in ballooned hepatocytes in zone 3, and mainly consist of cytokeratins 8 and 18, ubiquitin, and p62<sup>[37,38]</sup>. When MDBs are present in ballooned hepatocytes, they can be identified immunohistochemically as aggregates of cytokeratins 8 and 18 within otherwise cleared out cells<sup>[24]</sup>. However, it remains unclear whether MDBs serve as bystanders, exert protective effects, or promote injury<sup>[38]</sup>. The presence of MDBs is not definitively required, but is helpful for the diagnosis of NASH. MDBs are not specific to NASH; they are also observed in other liver diseases including alcoholic hepatitis, chronic cholestasis, and HCC<sup>[39]</sup>. In alcoholic hepatitis, MDBs are also observed in non-ballooned hepatocytes<sup>[8]</sup>.

### **Iron deposition**

Iron accumulation in NAFLD/NASH is usually mild, and can occur within hepatocytes, the sinusoidal lining cells of the reticuloendothelial system, or both<sup>[24,34,40]</sup>. Valenti *et al.*<sup>[41]</sup> reported that iron accumulation, which was predominant in hepatocytes but not in the reticuloendothelial system, was associated with advanced fibrosis in NASH. However, Nelson *et al.*<sup>[42]</sup> reported that NASH patients with iron accumulation in the reticuloendothelial system were more likely to have advanced fibrosis compared to patients with iron accumulation in hepatocytes. These results are contradictory, and further studies are needed to elucidate the significance of iron deposition in NASH.

### **Megamitochondria**

Megamitochondria are round or crystal-shaped eosinophilic structures in the cytoplasm of hepatocytes. They are observed most commonly in hepatocytes with microvesicular steatosis. Megamitochondria in NASH are distributed equally in all zones, and are abundant similarly in low- and high-stage groups<sup>[43]</sup>. Although megamitochondria are poorly understood lesions in NASH, they may be the result of injury from lipid peroxidation, or could represent an adaptive change<sup>[34]</sup>.

### **Other findings**

Ductular reaction refers to ductular proliferation at the portal tract interface. It arises from progenitor cells in the periportal area, and is accompanied by neutrophils and

stromal changes<sup>[34]</sup>. In NASH, the extent of ductular reaction is associated with fibrosis<sup>[44]</sup>.

One of the newly described lesions observed in NASH was the presence of an arterial branch in zone 3, commonly within perisinusoidal fibrosis<sup>[40,45]</sup>. Pathologists should maintain knowledge of this finding because it can be easily confused with the portal tract.

### **Pediatric NAFLD/NASH**

It is estimated that 2.6%-9.6% of children and adolescents have NAFLD<sup>[46-48]</sup>. Pediatric NAFLD/NASH has different histological characteristics than adult NAFLD/NASH<sup>[49]</sup>. Schwimmer *et al.*<sup>[12]</sup> investigated the histological features of 100 cases of pediatric NAFLD, and proposed that two different forms of steatohepatitis existed. Type 1 NASH had histological patterns usually seen in adult patients, and was characterized by steatosis, ballooning degeneration, and perisinusoidal fibrosis in the absence of portal features. Type 2 NASH exhibited a unique histological pattern in pediatric patients, characterized by steatosis, portal inflammation, and portal fibrosis in the absence of ballooning degeneration and perisinusoidal fibrosis. In their study, type 1 NASH was present in only 17% of subjects, whereas type 2 NASH was present in 51%, and the prevalence of simple steatosis was 16%. Advanced fibrosis was present in 8%, and cirrhosis was detected in 3%. In cases of advanced fibrosis, the pattern was generally comparable to type 2 NASH. Children with type 2 NASH were significantly younger and had a greater severity of obesity than children with type 1 NASH. Male patients were significantly more likely to have type 2 NASH and less likely to have type 1 NASH than female patients. Type 1 NASH was more common in Caucasian children, whereas type 2 NASH was more common in children of Asian, Native American, and Hispanic ethnicity. It is possible that there is a different pathogenesis, natural history, and responsiveness to treatments between type 1 and type 2 NASH. However, it remains unclear whether type 2 NASH converts into type 1 NASH with increased age of the patient.

Schwimmer *et al.*<sup>[12]</sup> characterized the histological pattern as an overlap of types 1 and 2 NASH or indeterminate in only 16% of subjects. However, subsequent studies reported that more than 50% of pediatric NAFLD cases exhibited overlapping features of types 1 and 2 NASH<sup>[50,51]</sup>. We compared the histological characteristics of NAFLD in 34 pediatric and 23 adult cases that were confirmed by liver needle biopsy<sup>[52]</sup> (Table 4). Steatosis was more severe in pediatric than adult cases, and half of pediatric cases presented with panacinar steatosis. Perisinusoidal fibrosis was significantly milder in pediatric cases than in adult cases, and lobular inflammation and ballooning were milder in pediatric cases compared to adult cases. In contrast, portal inflammation was more severe in pediatric cases. There were no obvious differences in the degree of periportal fibrosis between pediatric and adult individuals. Periportal fibrosis in the absence of perisinusoidal fibrosis (fibrosis stage 1C) was observed

**Table 4** Histopathological features of pediatric and adult nonalcoholic fatty liver disease cases *n* (%)

Item	Score/code	Pediatric ( <i>n</i> = 34)	Adult ( <i>n</i> = 23)
Steatosis			
Grade	0 (< 5%)	0 (0)	0 (0)
	1 (5%-33%)	10 (29)	7 (30)
	2 (> 33%-66%)	15 (45)	14 (61)
	3 (> 66%)	9 (26)	2 (9)
Location	Zone 3	12 (35)	14 (61)
	Zone 1	0 (0)	0 (0)
	Azonal	5 (15)	4 (17)
	Panacinar	17 (50)	5 (22)
Fibrosis			
Perisinusoidal <sup>1</sup>	0 (none)	19 (56)	6 (26)
	1 (mild)	10 (29)	8 (35)
	2 (moderate)	5 (15)	9 (39)
Periportal	0 (none)	18 (53)	13 (57)
	1 (mild)	13 (38)	6 (26)
	2 (moderate)	2 (6)	2 (9)
	3 (severe)	1 (3)	1 (4)
	4 (cirrhosis)	0 (0)	1 (4)
Stage	0 (none)	12 (34)	6 (26)
	1A (mild, zone 3, perisinusoidal)	4 (12)	5 (22)
	1B (moderate, zone 3, perisinusoidal)	2 (6)	2 (9)
	1C (periportal)	7 (21)	0 (0)
	2 (perisinusoidal + periportal)	6 (18)	5 (22)
	3 (bridging fibrosis)	3 (9)	4 (17)
	4 (cirrhosis)	0 (0)	1 (4)
Inflammation			
Lobular	0 (no foci)	7 (21)	2 (9)
	1 (< 2 foci per 200 × field)	18 (52)	13 (56)
	2 (2-4 foci per 200 × field)	7 (21)	6 (26)
	3 (> 4 foci per 200 × field)	2 (6)	2 (9)
Portal	0 (none)	11 (32)	10 (43)
	1 (mild)	14 (42)	11 (48)
	2 (moderate)	9 (26)	2 (9)
	3 (severe)	0 (0)	0 (0)
Ballooning	0 (none)	16 (47)	6 (26)
	1 (few)	10 (29)	13 (57)
	2 (many/prominent)	8 (24)	4 (17)
Diagnosis	Not NASH	8 (24)	4 (17)
	Borderline zone 1 NASH	7 (21)	2 (9)
	Borderline zone 3 NASH	5 (15)	5 (22)
	Definite NASH	14 (40)	12 (52)

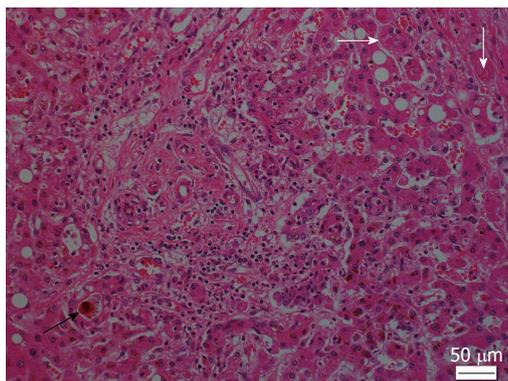
<sup>1</sup>Perisinusoidal fibrosis was significantly milder in pediatric cases than in adult cases. Borderline zone 1 NASH corresponds to type 2 NASH<sup>[52]</sup>. NASH: Nonalcoholic steatohepatitis.

exclusively in pediatric cases. The prevalence of bridging fibrosis and liver cirrhosis was lower in pediatric patients compared with adults. Type 2 NASH was observed in 21% of pediatric subjects, which was more than double the prevalence observed in adult subjects (9%). Fifty percent of pediatric cases exhibited overlapping features of types 1 and 2 NASH, and intralobular and portal changes were positively and significantly correlated. Our study, performed in Japanese individuals, suggested that pediatric NAFLD exhibits histological features that differ from those of adult NAFLD. The classification of “type 1 NASH” and “type 2 NASH” may be impractical as many pediatric NAFLD cases show overlapping features of types 1 and 2 NASH, and intralobular and portal changes are positively correlated. Recently, it was demonstrated that liver biopsy specimens from children with NAFLD and normal or mildly elevated alanine aminotransferase

(ALT) levels showed significant histological abnormalities, including advanced fibrosis in children with mildly elevated ALT<sup>[53]</sup>. Thus, measuring ALT may underestimate liver injury in pediatric NAFLD.

## HISTOLOGICAL DIFFERENCES BETWEEN ALCOHOLIC LIVER DISEASE AND NAFLD

The differential diagnosis of alcoholic liver disease and NAFLD is generally based on clinical information regarding alcohol intake, since differentiating based on histological appearance is challenging. However, several histological differences have been reported. Features more common in ALD than NASH include canalicular cholestasis, numerous and well-formed MDBs, prominent ductular reaction, and acute inflammation and fibro-



**Figure 5** Histological appearance of alcoholic steatohepatitis. Canalicular cholestasis (black arrow), Mallory-Denk bodies (white arrows), and acute inflammation in the portal tract are observed (hematoxylin and eosin staining).

sis in the portal tract (Figure 5). Sclerosing hyaline necrosis and veno-occlusive lesions are observed occasionally in alcoholic liver disease, but these lesions have not been reported in NAFLD<sup>[8]</sup>. In general, necroinflammatory activity in ASH is more severe than in NASH<sup>[27]</sup>. Features that are more common in NASH than ASH include severe steatosis, glycogenated nuclei, and lipogranulomas. Although steatosis is an important pathological feature for the diagnosis of NASH, it is not always present in ASH. Nakano *et al.*<sup>[54]</sup> reported that the fibrosis in NASH shows a lattice pattern, whereas fibrosis in alcoholic liver disease shows a solid pattern on reticulin-stained slides.

## INTEROBSERVER VARIATION IN THE HISTOPATHOLOGICAL ASSESSMENT OF NASH

The accurate evaluation of each pathological feature in NASH can be difficult, and thus accurate pathological diagnosis of NASH can be challenging. The significant difference in the prevalence of NASH between similar populations has been explained mainly by the different histological criteria used for diagnosing NASH in clinical studies<sup>[55]</sup>. As such, these findings made the results of clinical studies questionable. Therefore, we studied the extent of interobserver variation in the histopathological assessment of NASH<sup>[55]</sup>. In the study, eight hepatopathologists read liver biopsy slides of 21 cases where the clinical diagnosis was NASH or suspected NASH, and assessed the histopathological features. There was good agreement in evaluating the extent of steatosis and fibrosis, and moderate consistency concerning the localization of steatosis and fibrosis. However, there was only slight or poor agreement for evaluating ballooning and intra-lobular and portal inflammation. Two other studies also assessed interobserver variation in the histopathological assessment of NASH, and the findings were generally similar to our study, with the exception of good agreement for ballooning<sup>[14,56]</sup>. Establishing a standardized pathological diagnosis for NASH is necessary based on

the results of these studies.

## VALUE OF LIVER BIOPSY IN NAFLD/ NASH

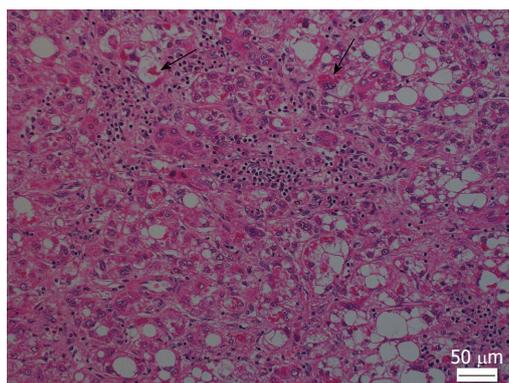
Liver biopsy is invasive and potentially harmful. Furthermore, histological lesions of NASH are unevenly distributed throughout the liver parenchyma, and liver biopsy sampling error can result in substantial misdiagnosis and staging inaccuracies<sup>[57]</sup>. Since liver biopsy has such drawbacks, novel imaging and serum-based assays to predict the presence of NAFLD/NASH, fibrosis, and/or inflammation have been studied.

Transient elastography measuring liver stiffness has been reported to have high negative predictive value and modest positive predictive value for assessment of advanced fibrosis in NAFLD; thus, it may be useful as a screening test to exclude advanced fibrosis<sup>[58]</sup>. Various serum molecular markers have been reported to be useful in diagnosing NASH or predicting fibrosis in NAFLD. The level of serum cytokeratin 18 fragments reflects hepatocyte apoptosis, and the area under the receiver operating characteristic curve (AUROC) for NASH diagnosis was 0.83<sup>[59]</sup>. Serum levels of Mac-2 binding protein (Mac-2bp), a major fucosylated glycoprotein, were recently shown to be elevated in NASH patients, and the AUROC for predicting NASH was 0.816<sup>[60]</sup>. Furthermore, it was reported that the Enhanced Liver Fibrosis panel (ELF), an algorithm of serum-based tests, had an AUROC of 0.90 for distinguishing severe fibrosis, 0.82 for moderate fibrosis, and 0.76 for no fibrosis in NAFLD<sup>[61]</sup>.

Although non-invasive tests for the diagnosis of NAFLD/NASH have progressed, as discussed above, the accuracy of these tests is inadequate. Particularly, characterization of individuals who are in the early and middle stages of NASH remains beyond the scope of most of these types of test. Currently, liver biopsy is the only method to accurately evaluate the extent and pattern of steatosis, necroinflammation, and fibrosis, and confirm the diagnosis of NAFLD/NASH. Liver biopsy evaluation remains the standard against which other assays and clinical algorithms must be matched and validated, and thus the histopathological evaluation of biopsy specimens continues to be the gold standard for diagnosing NAFLD/NASH.

## SH-HCC

NASH can progress to liver cirrhosis and HCC. The 5-year incidence of HCC for cirrhotic NASH was reported to be 11.3%<sup>[62]</sup>, and a substantial proportion of HCC cases without hepatitis B or C viral infections are estimated to be derived from NASH. However, the pathological features of HCC derived from NASH have not been elucidated for extended period of time. In 2010, Salomao *et al.*<sup>[63]</sup> reported a distinctive histological variant of HCC that they termed SH-HCC. SH-HCC shows features that resemble non-neoplastic steatohepatitis, including large



**Figure 6** Histological appearance of steatohepatic hepatocellular carcinoma. Large droplet steatosis, ballooning of malignant hepatocytes, Mallory-Denk bodies (arrows), inflammation, and fibrosis are observed in tumor tissue (hematoxylin and eosin staining).

droplet steatosis, ballooning of malignant hepatocytes, MDBs, inflammation, and pericellular fibrosis (Figure 6). In a subsequent study, the same group reported that the prevalence of SH-HCC in all HCC cases was 13.5%<sup>[64]</sup>. Almost all cases of SH-HCC occurred in patients with underlying steatohepatitis. SH-HCC was diagnosed in 35.7% of HCC patients with either NASH or alcoholic liver disease, compared with only 1.3% of HCC patients with other chronic liver diseases. Subsequently, Jain *et al.*<sup>[65]</sup> reported that SH-HCC was much more common in cirrhotic NAFLD patients compared with those with alcoholic cirrhosis. Therefore, SH-HCC is thought to be strongly associated with underlying steatohepatitis, particularly NASH.

## CONCLUSION

In this report, we reviewed the pathological features of NAFLD/NASH. Pathological assessment remains the gold standard for diagnosis of this disease. Steatosis, lobular inflammation, and hepatocellular ballooning are the necessary components for the diagnosis of NASH, and pathologists must evaluate these findings correctly. However, interobserver agreement can be poor in regards to evaluation of intralobular inflammation and ballooning, and thus clearly defined criteria for assessing these findings should be established. When evaluation of intralobular inflammation is difficult, immunohistochemical staining for leukocyte common antigen (a marker of lymphocytes) and CD68 (a marker of histiocytes/Kupffer cells) may be beneficial. Immunohistochemical staining for cytokeratin 8/18 is useful to objectively evaluate hepatocellular ballooning. Pediatric NAFLD has different histological characteristics from adult NAFLD, and special attention is needed for diagnosis.

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